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Prof. Dr. Anne Valle Zárate

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# **DEVELOPMENT OF HIGH QUALITY NICHE PRODUCTS FROM LOCAL CHICKEN AND PIG GENETIC RESOURCES**

## **Dissertation**

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**Philipp Muth**

born in Waiblingen, Germany

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**Supervisor and Reviewer:** Prof. Dr. Anne Valle Zárate

**Co-Reviewer:** apl. Prof. Dr. Michael A. Grashorn

**Additional examiner:** Prof. Dr. Jörn Bennewitz

**Head of the Committee:** Jun.-Prof. Dr. Andrea Knierim

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## Abbreviations

a*	instrumental redness
b*	instrumental yellowness
BF	backfat thickness
BW <sub>0</sub>	body weight at birth
BW <sub>A</sub>	asymptotic body weight
BW <sub>POI</sub>	body weight at the point of inflexion
BW <sub>wk</sub>	body weight at week wk
CIE	Commission Internationale de l'Éclairage
CL	cooking loss
Da	Dalton
DFD	dark, firm, dry meat
DG <sub>max</sub>	maximum daily weight gain
DL	drip loss
DeoMb	deoxymyoglobin
FAO	Food and Agriculture Organization of the United Nations
FG	fast-growing chicken line
FGL	fast-growing light-skinned chicken line
FPP	filter paper press method
GM	<i>m. gluteus medius</i>
He	hemin
IMC	intramuscular collagen
IMF	intramuscular fat
JCS	Japanese Color Score
K	exponential decay of the initial specific growth rate
L	initial specific growth rate
L*	instrumental lightness
LEA	loin eye area

LSM	least squares mean
LTL	<i>m. longissimus thoracis et lumborum</i>
Mb	water soluble myoglobin
MetMb	metmyoglobin
MUFA	proportion of monounsaturated fatty acids
M/W	meat area relative to the wet area after applying the filter paper press method
OxyMb	oxymyoglobin
pH1	pH at 45 min postmortem
pH24	pH at 24 h postmortem
pHu	ultimate pH (pH at 24 h postmortem)
PiDu	crossbred of the swine breeds Piétrain and Duroc
POI	point of inflexion
P/S	proportion of PUFA relative to the proportion of SFA
PSE	pale, soft, exudative meat
PUFA	proportion of polyunsaturated fatty acids
RSD	residual standard deviation
SFA	proportion of saturated fatty acids
SGD	slow-growing dark-skinned chicken line
SG	slow-growing chicken line
SGL	slow-growing light-skinned chicken line
SM	<i>m. semimembranosus</i> (paper III and IV) or soluble myoglobin (paper II)
SW	slaughter weight
WHC	water holding capacity determined by the filter paper press method

# 1 General introduction

In the decades after World War II, a rapid process of industrialization and specialization of pig and chicken production in high-income countries took place. Technological innovations led to a change in production and breeding methods, and the productivity and efficiency of meat production were continuously improved (Steinfeld et al., 2006; Neeteson-van Nieuwenhoven et al., 2013). Focusing on a few breeds and selecting production-related traits led to genotypes<sup>1</sup> adapted to market demands. Overall, the industrialization of pig and chicken production was successful in supplying increasingly urbanized societies with affordable monogastric meat<sup>2</sup> (Neeteson-van Nieuwenhoven et al., 2013). In parallel, the globalization of agro-food supply chains fueled the trade of commodities and input goods over large distances, in particular the transshipment of soymeal as an essential feed component, as well as the interregional exchange of genotypes. In consequence, the traded volume of chicken meat amounted to 10 million tons in 2010, which is more than 20 times the quantity traded in 1970 (FAOSTAT, 2015). The figures for pig meat are less intriguing – some regions neither produce nor import pork due to religious and/or cultural reasons – but reveal the same trend. Approximately 4 million tons of pig meat was traded in 2010, while, in 1970, only 0.6 million tons was traded (FAOSTAT, 2015). However, the industrialization and specialization in pig and chicken production, and the globalization of the commodity chains also elicited unwanted trends or externalities, two of which will be briefly outlined below.

Firstly, the industrialization of production and the globalization of value chains reshaped the pig and chicken meat sectors. Nowadays, industrial pig and chicken production units are characterized by their large scale, market orientation and decoupling from the location(s) of feed production (Steinfeld et al., 2006). The “prime-moving” regions for the industrialization of pig and chicken production were North America and Europe, where the restructuring was already enforced by the 1950s. There, both the total number of farms and the number of small farms in particular declined, while the share of large-scale farms in terms of the value of production increased (de Haan et al., 2010). Similar observations have been made for transforming economies, for instance, Thailand and Brazil (de Haan et al., 2010). In addition, the pig and poultry meat industries tend to form geographic clusters. For instance, in 2012, Europe’s largest pork producer was represented by the Lower Saxony and North Rhine-Westphalia federal states of Germany,<sup>3</sup> which, together, produced more pig meat than Spain and about twice as much as Poland. In the formation of clusters, production units agglomerate where transaction costs can be reduced through the interconnectivity of producers with suppliers of inputs and downstream industries (Gerber et al., 2010). But, in most countries, restructuring and clustering contributed to a decline in agricultural populations, and the abandonment of pig and chicken production units, particularly in remote areas. This development is not restricted to high-income countries. As indicated before, industrialized pig and chicken production systems were increasingly established in transforming countries that experienced a rise in demand for meat and/or benefit from locational advantages for export-oriented production (e.g.,

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<sup>1</sup> Within this thesis, the term genotype is in some cases preferred over the term breed, because, in the production tier of pig and chicken meat production, instead of breeds, crosses of several strains are often utilized.

<sup>2</sup> In the period from 1970 to 2010, the quantity of pig and chicken meat produced globally per capita increased from 9.9 to 15.4 kg and from 3.5 to 12.6 kg, respectively, while the volume of pig and chicken meat traded increased from 2 to 4% and 4 to 13%, respectively (FAOSTAT, 2015).

<sup>3</sup> Both federal states produced a combined cold carcass weight of 3.6 million tons in 2012 (DESTATIS, 2013).

availability of cheap labor), and, nowadays, often outperform the prime-movers in terms of the quantity produced<sup>4</sup>. An example for the former is given by the pig production sector of Eastern and Southeastern Asia. There, the quantity of pork produced per capita has increased from 6.8 to 26.1 kg over the last 50 years, with its peak growth in the 1980s along with the commercialization of the feed sector in China. The latter case concerns broiler production in South America, which was a net importer of chicken meat in 1970, but revealed an export surplus of 2.5 million tons of chicken meat in 2010 (FAOSTAT, 2015). In response to the de-activation of farms in rural areas and the globalization of value chains, resulting in a shift of value addition toward the domains of processing and retail, counter-movements in food production emerged. Within such development initiatives, rural actors in both high-income and transforming economies (see van der Ploeg et al., 2012 for examples from the Netherlands, China and Brazil), have, to quote Verhaegen and Van Huylenbroeck (2001, p. 444), “[...] been searching for new production and marketing models for quality foods in an attempt to reconcile the interests of producers (creating added value), consumers (food quality and health) and citizens (environmental and local development concerns).” Some of these production and marketing models have also been observed in the pig and chicken meat sectors.

Secondly, the genetic resource base of the pig and chicken sector was considerably affected by transitions in production and trade. Under the pressure of competition, a marked erosion of breed<sup>5</sup> diversity within the domestic pig and chicken was observed. Regional-specific, traditional breeds with inferior performance were quickly replaced by those that had been genetically improved according to the requirements of industrial production systems and, thus, were more profitable in these systems (Tisdell, 2003). In 2007, the FAO categorized 33 pig breeds and 101 chicken breeds as “international transboundary” breeds, representing the globally dominating breeds (FAO, 2007). The chickens used for meat production (broilers) result from crossbreeding schemes involving several purebred strains. The purebred strains are the property of a number of companies, namely Aviagen Broiler Breeders (owned by the EW Group, Germany), Hubbard (owned by Groupe Grimaud, France), and Cobb-Vantress (a subsidiary of Tyson Foods Inc., USA) (modified from Hiemstra and ten Napel, 2013). Besides those genotypes suitable for industrial production systems, the breeding companies recently expanded their portfolios, including slowly growing lines for organic and free-range production systems. Although to a lesser extent, this concentration of genetic resources in the hands of only a relatively few actors also applies to the pig breeding sector. According to Dekkers et al. (2011), the market share of the five largest breeding companies amounted to 23% in 2008 and resulted in a decline in the importance of individual breeders and breeders’ associations (Notter, 1999). Still, the diversity of documented breeds for the domesticated pig and chicken is rich, with 739 and 1,273 breeds worldwide, respectively (note: extinct breeds are included in the figures) (FAO, 2007). Looking more closely at the “prime-moving” regions of livestock industrialization, Europe and

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<sup>4</sup> The translocation of production is particularly notable for chicken meat production. Whereas in 1990 seven out of the top ten producing countries were high-income countries, four of which were European, in 2010, seven of the top ten chicken meat producing countries were lower- to upper-middle income countries. This meant that a European country was no longer ranked among the top ten. In the pig sector, China was the only upper-middle income country among the top ten producers of pork in 1990, but was joined by Brazil (upper-middle) and Vietnam (lower-middle) by 2010 (FAOSTAT, 2015).

<sup>5</sup> According to FAO (1999, p. 5), a breed is defined as “either a subspecific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other similarly defined groups within the same species or a group for which geographical and/or cultural separation from phenotypically similar groups has led to acceptance of its separate identity.”

North America, FAO reported 18 pig breeds and 46 chicken breeds as “regional transboundary” breeds, i.e. breeds that only occur in one of both regions, but in more than one country, and 183 local pig breeds and 680 local chicken breeds, where “local” means that these breeds occur only in one country (FAO, 2007). On the other hand, 124 pig breeds and 35 chicken breeds have become extinct (FAO, 2007). Thus, in both regions, a share of more than one third of the pig breed inventory is irrecoverably lost. Because farmers of transforming societies or those in the peri-urban areas of developing countries often adopt genotypes provided by the prime-moving regions due to the “path dependence” of such societies (Swanson, 1994 cited in Tisdell, 2003, p. 368), a further loss of local breeds can be expected. Indeed, in Asia, the application of unsystematic crossbreeding of local pig breeds with “international transboundary” genotypes imposes an additional threat to pig breed diversity (FAO, 2007), even where industrial livestock systems have not been (fully) implemented (Berthouly-Salazar et al., 2012). In the chicken sector, the situation appears to be less tense. This could relate to the lower maintenance costs of chicken populations, which appear to not exceed the capacities of hobbyists. In this way, the risk of extinction of local chicken breeds kept in high-income countries is, on the one hand, reduced, but, on the other hand, to date, most of these breeds have lost their economic relevance. In developing countries, local livestock and poultry breeds fulfil important roles in addition to commodity production, in particular for rural smallholders. They contribute to household subsistence, provide manure, generate savings, and have profound socio-cultural functions. In this situation, local breeds adapted to specific production systems remain highly relevant assets and can potentially contribute to poverty reduction (FAO, 2007). Where local pig and chicken breeds neither possess vital functions for their keepers nor are protected by market forces, they nevertheless possess values that could be worth conserving (see Mendelsohn, 2003 for detailed argumentation). Besides the value of pure existence, local breeds form a genetic stock represented by their pool of genes, encoding for traits such as disease resistance, specific product qualities, and resistance to drought, which could be combined with newly formed (synthetic) breeds or crossbreds in response to future challenges (Nimbkar et al., 2008). In addition, some local (pig) breeds play important roles in the maintenance of landscapes, for instance of the “Dehesa” – an agro-sylvo-pastoral system in southwestern Europe –, or possess a cultural heritage value. While there are different conservation strategies available in order to maintain a given genetic resource,<sup>6</sup> including methods of *ex situ in vivo* and *in vitro* conservation, only *in situ* conservation, i.e. the “[...] conservation of livestock through continued use by livestock keepers in the production system in which the livestock evolved or are now normally found and bred” (FAO, 2007, p. 443), allows for the preservation of knowledge and cultural values associated with the respective breed and artisanal techniques (Gandini and Villa, 2003; Lauvie et al., 2014). Thus, there are arguments in favor of conserving local pig and chicken breeds, particularly where they can be considered as a common good of a specific territory (Lauvie et al., 2013), and there is the need to contemplate “sustainable utilization” as the preferred way of doing so.

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<sup>6</sup> Besides “wild and feral populations, landraces and primary populations, standardized breeds, selected lines,” the definition of farm animal genetic resources by FAO (1999, p. 5) includes “any conserved genetic material,” such as cryoconserved gametes, embryos or somatic cells (FAO, 2007, pp. 461-466).

Therefore, the industrialization and specialization of pig and chicken production, and the globalization of agro-food chains have been, and still are, important evolutions within the agricultural sector. They were successful in providing affordable meat for urbanizing societies. Advances in technology and breeding in combination with adequate policies have the potential to mitigate some of the externalities that have accrued so far, for instance environmental and animal welfare issues (Neeteson-van Nieuwenhoven et al., 2013). But, firstly, the de-activation of pig and chicken meat production in rural areas, and, secondly, the loss of breed diversity within the domestic pig and chicken, demand “out of the box” approaches in order to provide development perspectives for primary producers and for the conservation of animal genetic resources and the values they bear. Hence, this study seeks to explore the potential for (re)valorizing pig and chicken genetic resources, determining the meat color and quality characteristics and their genotypic foundation, which could result in the uniqueness of local breeds. Where product characteristics were detected that could serve specific consumer needs, the feasibility of conserving the given pig and chicken genetic resource within alternative production systems and/or developing rural food networks is discussed.

### **1.1 High quality products from local animal genetic resources for niche marketing**

The industrialization of agricultural production and the globalization of food supply chains resulted in increased competition and restructuring within the livestock sector, the standardization of products, and price asymmetries in favor of processors and retailers. These changes finally contributed to the de-activation of farms and the decline of the agricultural population, and were of particular concern for the pig and chicken meat sectors, where scale effects allowed the widespread industrialization of production. On the contrary, increasing purchasing power, food scares, and criticism of production methods elicited differentiated consumer demands and shifts towards “more ‘local’, ‘alternative’ or ‘traditional/specialty’ foods [which are] typically constructed around notions of quality, territory and social embeddedness” (Ilbery and Maye, 2005, p. 332). Notably, the differentiation of consumer needs is not only evident in high-income countries, but, for instance, also in Vietnam, where affluent urban consumers are increasingly concerned with the safety of pork from intensive production and have expressed preference for pork from indigenous pigs that are fed “natural” diets (Lapar et al., 2010; Pedregal et al., 2010). The developments on both the supply and the demand side contributed to the (re)emergence of “alternative” food networks that are distinguishable from the global commodity chains by the type of linkages between producers and consumers, and the practices of food provision (Roep and Wiskerke, 2012). Because, these alternative food networks are often based on the reproduction and (re)valuation of local sources providing “food of distinct and better appreciated qualities” (Roep and Wiskerke, 2012, p. 206), they have been considered as appropriate rural development strategies in order to reduce the market risk, particularly for small- and medium-sized farms (Ilbery et al., 2004; Honeyman et al., 2006). In the following section, the peculiarities of such food networks with respect to high quality pig and chicken meat are briefly elucidated.

In some countries, notably France, with its long-established “Label Rouge” scheme, and Italy, with its outstanding culinary traditions, such quality food networks always coexisted besides the commodity market, while in other countries, pressures through competition and changing consumer demands created a framework that stimulated the development of new networks (van der Ploeg et al., 2012). In spite of the fact that, in some instances, expectations were too high with respect to the economic impact and viability of such initiatives (Verhaegen and Van Huylenbroeck, 2001; Ilbery et al., 2004), they were frequently supported by rural development policies, for example, the Common

Agricultural Policy (covering several legislative texts) as well as the Protected Designation of Origin (PDO) and the Protected Geographical Indication (PGI)<sup>7</sup> policies of the European Union (Regulation (EU) No 1151/2012). Newly emerging markets and networks have been analyzed using different approaches, highlighting aspects of economics, marketing and/or sociology, which led to slightly different notions. Expanding the definition of alternative food networks given by Roep and Wiskerke (2012), van der Ploeg et al. (2012, p. 140) considered “nested” markets to be specific segments of mainstream markets, but distinguishable through a price differential and a different distribution of added value along the supply chain, as well as by distinct infrastructural and governance patterns. In contrast to van der Ploeg et al. (2012), Toften and Hammervoll (2013) argued that niche markets have to be considered as independent from mainstream markets. They defined niche marketing as “the process of carving out, protecting and offering a valued product to a narrow part of a market that displays differentiated needs” (Toften and Hammervoll, 2013, p. 280). Both approaches intersect with respect to the added value of the product created. Thus, generating added value or, in other words, differentiated quality, is a central aspect of nested or niche markets.

The strength and resilience of nested markets is founded on so-called “common-pool resources”. This term was addressed by van der Ploeg et al. (2012),<sup>8</sup> and describes a normative framework that is commonly shared by producers and consumers, regulating the elaboration and distribution of products. Common-pool resources are also reflected in the recognition of the special quality of a product by consumers, and the common understanding of the definition of quality by the stakeholders within a nested market (van der Ploeg et al., 2012). In an alternative food network where, for instance, the choice of breed is prescribed by producers’ regulations and where the higher (perceived) quality of the products is appreciated by (a group of) consumers, a breed can be considered as a common-pool resource. Therefore, embedding high quality products and/or distinctive products in niche markets could open up possibilities for the conservation of local breeds (Ligda and Casabianca, 2013), in particular where, such as in pig and chicken meat production, only a few highly productive international transboundary breeds dominate (FAO, 2013).

Given that high (perceived) product quality is an essential feature of nested markets or niche markets, how can unique and differentiated product quality be understood, in particular with regard to pig and chicken meat production? Approaching this question for food products in general, Grunert (2005) summarized four topics that form food quality from a consumer’s point of view: taste and other sensory characteristics, health, convenience, and process-related characteristics, such as organic or animal friendly production. Most health- and process-related qualities represent credence qualities, which, by definition, cannot be evaluated by the consumer even after consumption of the product. Claims on the locality of production also fall into this category. Thereby, a product is linked to its geographical origin or to cultural significance, i.e. provided with value-laden information (Ilbery and Maye, 2005; FAO, 2013). In Europe, a number of multifaceted alternative pork production systems that assert (combinations of) claims on eating quality, animal welfare, environmental impact, locality, nutritional quality, and organic production have evolved (Bonneau and Lebret,

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<sup>7</sup> In 2010, 110 different fresh meat products held a geographical indication (PDO or PGI) according to Council Regulation (EC) No 510/2006 (now replaced by Regulation (EU) No 1151/2012), accounting for a sales value of 1.2 billion Euro (Annex to Chever et al., 2012).

<sup>8</sup> Interestingly, the definition of “common-pool resources” is reflected well in the “internal dynamic capabilities” of niche marketers, defined by Toften and Hammervoll (2013, p. 281), and the “embeddedness” of alternative food networks, defined by Roep and Wiskerke (2012, pp. 208-209).



2010). Furthermore, in chicken production, systems differentiated from conventional production have been established, for instance the aforementioned Label Rouge<sup>9</sup> scheme in France, or the farms certified according to the “Tierschutzlabel”<sup>10</sup> in Germany. Eating quality and claims on local production are frequently combined in alternative pig and chicken production systems, suggesting a potential to create added value through these features within niche production and marketing. Therefore, in the following, this relationship is further discussed.

The four previously mentioned quality spheres create an intermediate concept of quality in the minds of consumers (Grunert, 2005). In retail situations, consumers aim at maximizing “value for money”, trying to get the best quality at the lowest price (Grunert, 2005). Because many intrinsic quality cues, i.e. those attributes physically linked to the product, are not available for evaluation at purchase, consumers substitute them by extrinsic quality cues, i.e. “any product characteristics that can be altered without influencing the objective nature of the product or service” (Veale and Quester, 2009, p. 195). These cues contribute to the quality expectation of customers, are of high importance for food choice, and can even overrule intrinsic characteristics. The most prominent extrinsic quality cue is the price, but social and credence attributes also belong to this category. During consumption, the perception of the quality of the product is formed through general integration of all influential factors in the brain, including sensory characteristics, but also physiological, behavioral and cognitive factors (Imram, 1999). Thereby, sensory quality can, according to Deliza and Gloria (2009, p. 526), be defined by “adequate levels of sensory attributes considering the appearance, aroma, flavor, and texture”. Sensory characteristics can be further classified into anticipatory and participatory attributes. In addition to extrinsic cues, anticipatory attributes, such as appearance, enable assessment of product quality at purchase, whereas participatory attributes, such as aroma, flavor and texture, are only available during consumption (Imram, 1999), and can be subsumed as “eating quality”. Thus, because the consumers’ context and background (e.g., gender, culture, age and physiological state) and the expectations arising from extrinsic quality cues substantially contribute to the subjective quality perceived by the consumer within the experience of the product (Imram, 1999), the concept of (high) quality food has a much wider scope than sensory or eating quality alone.

Nevertheless, the sensorial distinctiveness and organoleptic superiority of products are important assets, particularly in the development of marketing strategies for breed-specific niche products (FAO, 2013; Lund, 2010 cited in Ligda and Casabianca, 2013). FAO (2013) claims that value adding strategies require an objective evaluation of factors that will enhance demand, including product quality and distinctiveness, and, as a result, allow the valorization and traceability of the product. However, the assessment of sensory quality attributes by taste panels is challenging given the numerous factors which influence the subjective perception of quality, and because of the high costs that incur. Sensory attributes relate to technological – in the sense that they can be controlled and

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<sup>9</sup> The Label Rouge scheme was developed in France in the 1960s and has been approved by the French authorities. It is a production method based on access to free range and primarily makes claims on animal welfare, environmental protection, and the superior organoleptic quality of meat (Syndicat National des Labels Avicoles de France, 2013).

<sup>10</sup> The “Tierschutzlabel” is a label for pig and chicken meat producers developed by the German Association for Animal Protection (Deutscher Tierschutzbund e.V.). It primarily raises a claim on animal welfare through the regulation of production, transport and slaughter. The genotypes applied for chicken meat production must not exceed an average daily gain of 45 g/day (Deutscher Tierschutzbund e.V., 2013).

manipulated – and/or functional product specifications (see, for instance, Nam et al., 2009 and Moeller et al., 2010 for relationships between sensory evaluations and physico-chemical pork quality). The establishment of technological quality specifications thus enables the measurement and documentation of the physico-chemical characteristics of breed-specific products, which is essential for evaluating the market-conformity of the produce (González et al., 2014), and for genetic breed improvement and the development of (niche) marketing strategies (FAO, 2007; Tixier-Boichard et al., 2008; Lund, 2010 cited in Ligda and Casabianca, 2013). The technological or functional quality of meat additionally reflects the suitability of meat for further processing, storage and retail display (Rosenvold and Andersen, 2003), and is characterized by measurable properties such as water holding capacity, color, fat content and composition, oxidative stability, and uniformity.

Thereby, meat color, objectively measurable through colorimetry, takes an exposed position within the complex of meat quality, because this trait primarily contributes to the appearance<sup>11</sup> of meat products and is one of the few intrinsic quality cues which can be evaluated at purchase, allowing for the discrimination of products on display.

Thus, basing new food networks on the higher (perceived) quality or distinctiveness of its product(s) could open possibilities for the *in vivo in situ* conservation of local breeds. While sensory attributes form only a part of the “Total Food Quality Model” (Grunert, 2005, p. 373), they are nevertheless crucial for the development of marketing and genetic breed improvement strategies. Therefore, with respect to meat products, the documentation of breed-specific technological and/or functional quality product traits is a first essential step in order to position and protect product(s) on the market by differentiation from commodity produce, and thus safeguards the economically sustainable utilization of pig and chicken genetic resources (Ligda and Casabianca, 2013).

## **1.2 Effects of selection on high yield regarding meat color and quality of pigs and chickens**

The efficiency of growth in meat producing species depends on the time elapsed and the feed consumed until reaching market weight relative to the value per weight unit (Dickerson, 1970). The gain of body tissue in a short time is therefore crucial for the profitability of meat production. Because, in most cases, animals are slaughtered before they reach maturity, the growth rate is more important than the maximum attainable body weight and/or size at maturity. The skeletal (striated) musculature of slaughter animals – their meat – is the most valuable component of the carcass and determines the value per weight unit of the slaughtered animal. This explains why reducing the dorsal fat depth and the abdominal fat pad in pigs and chickens, respectively, were soon incorporated into breeding goals, which were, until the 1990s, strongly geared toward improving productivity. Over time, the breeding objectives were broadened and, to date, also include trait complexes related to the environmental impact of production, animal health and welfare, product quality, food safety, and genetic diversity (Neeteson-van Nieuwenhoven et al., 2013). However, productivity still has a high, if not the highest, weight within the overall breeding goals. The intensive genetic selection of the domestic pig and chicken and systematic crossbreeding schemes have led to animals that are clearly superior in terms of body size and growth rate. Compared to their wild

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<sup>11</sup> According to Lozano (2006, p. 166), appearance is defined as “psychophysical property of materials or objects interacting with light, provided by a specific light source in a defined mode (spatial and spectral composition) and being detected, observed and perceived by human beings at a defined spatial position with respect to them.”

ancestors, broiler chickens and domestic pigs revealed a markedly increased gain in body weight (Jackson and Diamond, 1996; Rehfeldt et al., 2008). But, there were also drastic differences in daily weight gain and muscularity comparing modern broilers to random bred chicken lines (Havenstein et al., 2003; Schmidt et al., 2009) and comparing rustic British (Berkshire and Tamworth) pig breeds to modern breeds (Large White and Duroc) (Wood et al., 2004). Taking into account that the nutritional quality of diets improved over the last decades, Wood et al. (2004) and Havenstein et al. (2003) additionally used diet formulations not optimized according to the requirements of modern pig and chicken lines, respectively. However, the modern genotypes still outperformed the traditional, less intensively selected breeds suggesting a marked genetic effect on the growth and yield of lean tissue relative to the effect of nutrition (Havenstein et al., 2003; Wood et al., 2004). In the following section, the underlying structural and physiological mechanisms responsible for the advances in lean tissue gain of the domestic pig and chicken, and their relationship to meat color and quality will be introduced.

### **1.2.1 Structural and physiological changes in skeletal muscle induced by selection on performance traits and their relationship with the meat quality parameters of pigs and chickens**

Different muscle types within an organism and homologous muscles of different species show a marked variation in the rate and magnitude of tension development, power output, shortening velocity, and resistance to fatigue. At the same time, meat color and quality vary substantially within individuals and between species, indicating that muscle function and the expression of meat quality traits are somehow related. Indeed, muscles exhibiting different metabolic profiles vary in their susceptibility to meat defects. So-called “white” muscles are more prone to the development of pale, soft, exudative (PSE) meat, whereas “red” muscles are more likely to develop dark, firm, dry (DFD) meat (Eikelenboom et al., 2004). Muscles are primarily composed of muscle cells (myofibers) and differ in the total number of myofibers and the size (length and cross-sectional area) of myofibers (Rehfeldt et al., 2004), which is a result of an altered protein turnover, i.e. a changed balance of anabolic and/or catabolic processes. The superior muscle mass and yield of selected pig and chicken genotypes are thus based on the manipulation of the principles of growth, that is, hyperplasia (increase in the number) and/or hypertrophy (increase in the thickness) of myofibers (Rehfeldt et al., 2004). In chickens, broiler embryos showed an increased myoblast proliferation during *in ovo* development, resulting in a larger total number of myofibers, while the cross-sectional myofiber area in their pectoral muscle was reduced compared to layer embryos (Al-Musawi et al., 2011). However, while the number of myofibers remained constant posthatch, the radial myofiber size in chicken pectoral muscles grew considerably within the first weeks of age (MacRae et al., 2006). The increased hypertrophy of fast-growing broiler chickens also quickly resulted in a larger myofiber cross-sectional area in the breast muscle compared to slow-growing lines (Burke and Henry, 1997; Scheuermann et al., 2004; MacRae et al., 2006). In line with these findings, Le Bihan-Duval et al. (2008) demonstrated that within a Hubbard sire line, both body weight gain and breast muscle yield were genetically correlated to the myofiber cross-sectional area with coefficients of 0.69 and 0.48, respectively. Furthermore, comparing chicken genotypes differing in breast muscle yield, the frequency of myofibers with cross-sectional areas  $\geq 3000 \mu\text{m}$  increased for high yielding chickens, whereas the frequency of fibers with cross-sectional areas of  $\geq 1000$  to  $< 2000 \mu\text{m}$  and  $< 1000 \mu\text{m}$  increased for a standard breast yield genotype (Petracci et al. 2013). In pigs, the increased muscle mass of domestic German Landrace pigs compared to wild boar specimens was mainly related to myofiber hypertrophy, whereas the average cross-sectional myofiber area in the longissimus dorsi muscle, at

an age of 20 weeks, more than doubled in growing domestic pigs compared to wild boar (2880 vs. 1300  $\mu\text{m}$ ) (Rehfeldt et al., 2008). In contrast, the differences in myofiber number were less pronounced and depended on the muscle under investigation (Rehfeldt et al., 2008; Lösel et al., 2013). For purebred Large White pigs, the mean cross-sectional area of myofibers was genetically related to the average daily gain, lean meat percentage and loin eye area, exhibiting genetic correlation coefficients of 0.29, 0.41 and 0.64, respectively (Larzul et al., 1997). While the total number of myofibers did not reveal clear genetic associations to growth and muscularity in the previous study (Larzul et al., 1997), the total number of myofibers in the longissimus lumborum was genetically positively related to live weight (0.44) and loin eye area (0.38) in four German pig breeds (Fiedler et al., 2004). In their study, Fiedler et al. (2004) found a low negative genetic correlation between average myofiber cross-sectional area and live weight (-0.16), while the average myofiber area genetically correlated positively to loin muscle area (0.22).

Thus, structural alterations of skeletal muscle underlie genetic advancement in several growth-related traits. In addition, a number of analyses of the genetic association of myofiber number and cross-sectional area and meat quality traits have been published for both pigs and chickens. For a grandparental chicken line (Hubbard), correlations of myofiber size with meat quality characteristics of the chicken breast muscle have been reported by Berri et al. (2007). Thereby, an enlarged cross-sectional area was genetically and phenotypically negatively related to the glycolytic potential, i.e. the remaining glycogen level was reduced. Due to the fact that, after death and under anoxic conditions, ATP is synthesized via the anaerobic or glycolytic pathway, converting the glycogen stores to lactate and  $\text{H}^+$  (Lefaucheur, 2010), lower initial glycogen levels limit the extent of acidification. Consequently, the myofiber cross-sectional area was negatively related to lactate accumulation and positively to the pH values assessed at 15 min and 24 h postmortem. Meat lightness decreased when the myofiber cross-sectional area increased. On the phenotypic level, the cross-sectional area was not correlated to the yellowness of the breast muscle, but a negative genetic correlation between these traits was detected. Drip loss was reduced in the breast muscles of chickens exhibiting enlarged cross-sectional fiber areas at phenotypic and genetic correlation coefficients of -0.23 and -0.44, respectively (Berri et al., 2007). For Large White pigs, Larzul et al. (1997) found positive genetic correlations of average myofiber size with the ultimate pH, intramuscular lipid percentage and pigment concentration. With respect to the ultimate pH value, the opposite was found by Fiedler et al. (2004), who reported a negative genetic relationship between myofiber cross-sectional area and pH, but a positive genetic correlation between myofiber cross-sectional area and drip loss for four different pig breeds. Additionally, the frequency of giant fibers was genetically negatively correlated to pH and, correspondingly, positively to drip loss (Fiedler et al., 2004). These results suggest that alterations of hyperplasia and hypertrophy could well correspond to changes in the physico-chemical quality of monogastric meats.

The specific myofiber composition is responsible for the diversity of functional properties among muscle types, resulting in the muscle-specific contractile and metabolic profile. Myofiber types differ according to the quantitative regulation of gene expression and the polymorphism of the myosin heavy chain (Reggiani and Mascarello, 2004). In the skeletal muscle of the pig, four myosin heavy chain isoforms can be differentiated by the use of monoclonal antibodies, which, listed in the order of the speed of contraction, result in the identification of four myofiber types: I < IIa < IIx < IIb. Myosin is a large, hexamere molecule that transforms small conformational changes, which result from the hydrolysis of ATP, into larger filamentous movements, causing the contraction of muscles

(Reggiani and Mascarello, 2004). Each myosin heavy chain isoform is encoded by a single gene and expressed together with specific myofibrillar proteins (thick and thin filament proteins as well as Z line proteins), resulting in variation in the speed of contraction/relaxation, in the sensitivity to calcium ions, and in the metabolic properties of myofibers (Reggiani and Mascarello, 2004; Lefaucheur, 2010). Using histochemical classification, myofibers are commonly grouped as slow-twitch oxidative, fast-twitch oxido-glycolytic and fast-twitch glycolytic, corresponding to I, IIa and IIx and/or IIb myofibers, respectively, whereas the latter two cannot be properly distinguished by histochemistry (Reggiani and Mascarello, 2004; Lefaucheur, 2010). The most economically important muscle of the chicken, the breast muscle, predominantly consists of a single fast-twitch glycolytic myofiber type. Although small proportions (<4%) of fibers of a slow-twitch myofiber type have been detected in the pectoral muscle of slow-growing lines (Branciani et al., 2009; Zhao et al., 2012), it can be assumed that the role of myofiber composition is of lesser importance for the expression of meat quality traits of this muscle. In contrast, major importance for the functional and eating quality of pork has been attributed to the myofiber composition of pig muscles. In porcine muscles, myofibers are arranged in a typical way, whereas islets of slow-twitch oxidative fibers are surrounded by fast-twitch oxidative fibers and, at the periphery, by fast-twitch glycolytic fibers (Reggiani and Mascarello, 2004). In the loin, fast-twitch glycolytic myofibers are the dominating type and, on average, occupy around 49.2-84.5% of the relative fiber area (Larzul et al., 1997; Chang et al., 2003). Other muscles exhibit considerably higher proportions of slow-twitch oxidative fibers, for example, the total fiber area of the dark part of the semitendinosus muscle was, on average, represented by 51.5% of type I fibers (Realini et al., 2013) and the total fiber area of the psoas major was on average represented by 33.9-41.6% of type I fibers (Chang et al., 2003). The domestication of pigs and the selection of production traits appear not only to have resulted in the increased number and size of myofibers, but also in an altered composition of myofiber types within muscles. Compared to wild boar, German Landrace pigs revealed an increased proportion of fast-twitch glycolytic fibers in the loin (Rehfeldt et al., 2008; Lösel et al., 2013). In a French Large White population, productive traits such as daily weight gain and lean meat percentage were genetically positively related to the cross-sectional area of the IIx and IIb fiber types (Larzul et al., 1997). The genetic analysis carried out by Larzul et al. (1997) revealed furthermore that an increase in the proportion of fast-twitch glycolytic fibers was associated with an augmented glycolytic potential, decreased pH values measured at 30 min and 24 h postmortem, and an increased lightness of the meat. Fiedler et al. (2004) also reported a negative genetic correlation regarding the proportion of fast-twitch glycolytic fibers in the longissimus lumborum and pH. However, comparing two modern pig breeds with two rustic British breeds, the differences in myofiber composition were inconsistent (Chang et al., 2003), despite the manifestation of marked differences in growth performance and leanness among the investigated breeds (Wood et al., 2004). Indeed, there is some evidence that, in the pig, a systematic relationship between myofiber type with myofiber cross-sectional area (Realini et al., 2013) and metabolic profile (Chang et al., 2003) does not exist. Additionally, variations in neuronal signaling patterns, exercise or hormonal stimulation could lead to postnatal modifications in the myofiber composition of a muscle (Reggiani and Mascarello, 2004). The myofiber composition and profile is, nevertheless, of concern regarding the expression of the meat color and quality of the pig. Chang et al. (2003) suggested that the superior meat quality of porcine psoas major muscle, which exhibits lower drip loss and increased tenderness, compared with the longissimus dorsi muscle was related to the higher abundance of type IIa and IIx fibers in the former. Gil et al. (2008) demonstrated that across different pig breeds an increased percentage of myosin heavy chain I isoforms and slow-twitch oxidative myofibers in the longissimus and semimembranosus muscles were associated with elevated redness

and pigment levels, while, in contrast, drip loss and lightness were positively correlated with markers of glycolytic activity. From their study on a population of commercial crossbreds (Landrace x Yorkshire x Duroc), Nam et al. (2009) concluded that myofiber size is mainly related to the color in both fresh and cooked pork and to abnormal flavor intensity, whereas myofiber composition is associated with fresh pork color and taste acceptability after cooking.

### **1.2.2 Between- and within-line differences in meat quality parameters of pigs and chickens selected for performance traits**

Thus, the selection on growth performance considerably induced altered properties of pig and chicken muscles, and there is some evidence that these changes correlate to a divergent expression of meat color and quality traits. Comparison of the meat color and quality of slow-growing lines to that of fast-growing lines could, therefore, be seen as a straightforward approach in order to determine changes in meat color and quality related to selection on growth efficiency. The slow-growing lines used for such comparative studies are frequently represented by traditional, less intensively selected purebreds and/or, in the case of the chicken, by layer strains. But, some considerations on this approach are necessary. As Griffin and Goddard (1994) pointed out, for the chicken, comparing different genotypes implies the comparison of different genetic backgrounds because different breeds are involved in the genetic make-up of modern genotypes and founder effects may exist. Thus, differences in meat quality between different genotypes may be due to other genetic factors than the differences elicited by selection on growth-related traits. The halothane gene of pigs could be taken as one example to illustrate this situation. The halothane allele leads to stress-susceptible pigs (Porcine Stress Syndrome) in homozygous animals, as well as to proneness to the development of the PSE defect of meat (Ciobanu et al., 2011). But, because of its additive and beneficial effect on carcass yields and lean percentages, production trait selection led to an increased incidence of the allele and the associated trade-offs in meat quality, particularly in the Piétrain population (Eikelenboom et al., 2004; Rehfeldt et al., 2004). Thus, while there is a clear association between production trait selection and decreased meat quality in the Piétrain, selection for dressing percentage or leanness may not be related to deterioration of meat quality in other breeds due to an absence or decreased allele frequency of the n allele. The main caveat for accepting different genetic backgrounds of fast- and slow-growing breeds applies to the vast majority of the experiments documented so far. This does not apply once the compared genotypes have been selected from the same base population. Such experiments, involving selected lines and unselected counterparts, are more seldom, but, although factors such as genetic drift could also bias the results here, they are of high interest for the evaluation of the impact of selecting for production traits on meat color and quality. Selection experiments have been conducted utilizing chicken strains by Berri et al. (2001) and pig breeds by Sonesson et al. (1998) and Oksbjerg et al. (2000). The study by Sonesson et al. (1998) did not include a comparative approach and is treated in short in a later paragraph. Another challenge for the comparison of meat quality attributes between genotypes exhibiting different growth performances is inevitably related to the growth rate itself. Because the growth rate is increased for the selected lines, their body weight at equal chronological age is higher than that of slow-growing lines. Depending on the study objective, there are, thus, roughly two types of experimental designs that can be distinguished: fast-growing genotypes slaughtered at a younger age compared to slow-growing genotypes, when opting for an equal body weight at slaughter, or at a much higher slaughter weight, when the age at slaughter is fixed. This bias is treated more in-depth in chapter 6.1.

This paragraph focuses on the recent selection experiments and their implications for the association of growth rate and meat quality. Oksbjerg et al. (2000) stated that selection of performance traits over 20 years, such as daily gain, feed conversion ratio and lean meat percentage, only slightly affected the meat quality traits of pigs. The study compared Danish Landrace pigs from the year 1995 to pigs of the same base population, which had been unselected since the mid-1970s, slaughtered at an age of 60 and 85 days, respectively. The difference in daily weight gain between the selected hogs and their counterparts amounted to  $\Delta 288$  g/day. Somewhat unexpected, the selected line revealed a reduced average cross-sectional area of myofibers in the longissimus dorsi, thus, the increased accretion of muscle mass in the Danish Landrace population could have been realized by a rise in the total number of muscle fibers. The proportion of fast-twitch glycolytic fibers in the longissimus dorsi of the selected line was increased at the expense of slow-twitch oxidative fibers. Yet, the postmortem glycolytic rate did not differ between both lines. Furthermore, the tenderness scoring was reduced and cooking losses were increased for the selected animals, while the augmented concentration of heme pigments in the loin chops of the unselected line resulted in significantly increased meat redness and decreased lightness. With respect to the elevated pigment contents, however, the older age at slaughter of the unselected line has to be noted. Other sensory or technological meat quality traits did not differ between the two pig populations (Oksbjerg et al., 2000). For selected and unselected chicken lines, each derived from two base populations, an experimental and a commercial one, Berri et al. (2001) detected an elevated glycolytic potential in the unselected lines and, correspondingly, an accelerated postmortem pH drop and lower ultimate pH values compared to the selected counterparts. The drip loss of breast meat during storage was, however, not affected. Additionally, the heme pigment content declined in the breast muscle of the selected birds. Alterations of both pH development and the concentration of heme pigment could have contributed to the marked line effects on meat color and color stability during storage (Berri et al., 2001). The differences in enzyme activities were rather small, which led to the conclusion that the metabolic pattern was not largely affected by selection. Although not representing a selection experiment, the multistrain comparison conducted by Sandercock et al. (2009a; 2009b) is an interesting approach to the topic. Comparing multiple broiler, layer and traditional strains, it was found that the final pH value of broiler chickens slaughtered at an age of 8 weeks was significantly reduced, which contradicts the results obtained by Berri et al. (2001). Breast meat redness and shear force of the broiler lines were decreased (Sandercock et al., 2009b). The broilers scored higher in a taste panel assessment for texture and overall liking when slaughtered at an age of 6 weeks, whereas, at a slaughter age of 10 weeks, the scoring for texture and overall liking did not differ between the strains (Sandercock et al., 2009a).

In addition to selection experiments, genetic analyses within individual breeds or lines are insightful with respect to the relationship between productive traits and meat color and quality, some of which are introduced here. For an experimental meat-type chicken line, a negative genetic association between body weight at 6 weeks and redness of the pectoral muscle was reported by Le Bihan-Duval et al. (2001). It was also indicated that selecting for an increased breast meat yield would result in decreased redness and yellowness of the meat, but no further alterations of meat quality were expected by genetically improving production of this genotype (Le Bihan-Duval et al., 2001). In accordance with this study, Chabault et al. (2012) reported a negative genetic correlation between body weight at slaughter and breast meat redness for a medium-growing line. Additionally, they discovered that breast yield was genetically negatively related to pH assessed at 15 min postmortem. For a Hubbard male grandparental line, genetic associations between breast muscle mass and

lightness, drip loss, thawing-cooking loss, and toughness were detected (Le Bihan-Duval et al., 2008); all of these correlations pointed in a favorable direction. For one elite flock of a male broiler line, no genetic relationship between meat quality traits and body weight were detected (Gaya et al., 2011). But, breast weight revealed a positive genetic correlation with the initial pH value and yellowness, and a negative genetic correlation with thawing-cooking loss (Gaya et al., 2011).

Some scientific reports on the genetic association of meat color and quality traits with performance traits for conventional pig breeds exist. The carcass weight and lean weight of Duroc and halothane-free Landrace pigs did not exhibit any significant genetic correlation with physico-chemical meat quality traits in a study by Cameron (1990). For Duroc and Landrace populations, Lo et al. (1992) observed a positive genetic association between growth rate and intramuscular lipid content, whereas growth rate was genetically negatively correlated to shear force. Furthermore, average daily gain was genetically favorably related to water holding capacity, even though the genetic relationship of growth rate with subjective juiciness was negative. Longissimus muscle area revealed largely unfavorable genetic correlations with pork quality traits, indicating that selection for increased loin eye area could result in reduced intramuscular fat content and water holding capacity, but increased moisture content and shear force (Lo et al., 1992). In contrast, the genetic correlations of the lean meat percentage of a Dutch Yorkshire population with pH, water holding capacity, and color were close to zero according to de Vries et al. (1994), whereas the genetic relationship of lean meat percentage with intramuscular fat content was negative. Growth rate showed no strong correlations with meat quality traits in the same study (de Vries et al., 1994). The lean meat percentage of Large White, Landrace and Piétrain in Austria was genetically negatively correlated to pH and positively to drip loss (Knapp et al., 1997). The genetic correlation of leanness with intramuscular fat ranged between 0.02 and -0.31 among the three breeds. A Large White population selected for weight gain exhibited a negative genetic trend for the ultimate pH, and positive genetic trends for both the initial pH and yellowness (Sonesson et al., 1998), but, when selecting for decreased backfat thickness, the corresponding changes in meat quality were less pronounced. Sonesson et al. (1998) pointed out that the lean meat percentage in particular had unfavorable genetic correlations with meat quality traits, especially adversely affecting water holding capacity. For Duroc, Suzuki et al. (2005) reported a negative genetic correlation between the average daily gain and toughness, and a positive genetic relationship between the average daily gain and meat lightness. The genetic correlation between growth rate and intramuscular lipid was weakly positive. The longissimus muscle area showed undesirable genetic correlations, particularly with drip loss (0.64), but also with intramuscular fat (-0.26) and toughness (0.23) (Suzuki et al., 2005). For the progeny of a synthetic halothane-free boar line and a commercial line, van Wijk et al. (2005) reported particularly highly negative genetic correlations of growth rate with the ultimate pH, and correspondingly strong positive genetic correlations of growth rate with meat lightness and drip loss. In contrast, the lean meat percentage exhibited no genetic association with the ultimate pH and water holding capacity, while instrumental color values of the loin were negatively correlated to the lean meat percentage (van Wijk et al., 2005). In Iberian pigs, an increase in primal cut weights was genetically related to a decrease in intramuscular fat content and the proportion of saturated fatty acids (Fernández et al., 2003). The share of linoleic acids in intramuscular lipids was particularly genetically positively related to the primal cut weight in this autochthonous European breed.

In summary, selection on growth performance had a profound impact on (micro)structural and physiological properties and the composition of muscles. Some of these changes may be coupled



with meat quality attributes and could result in altered meat color and quality of breeds, which were less intensively selected, compared to those genotypes that dominate pig and poultry meat production.

### **1.3 Objectives and structure of the thesis**

The framework for this thesis is based on the restructuring of the pig and chicken meat sectors and the abandonment of pig herds and chicken flocks on farms in many rural regions on the one hand, and, on the other, the decline in pig and chicken breed diversity due to the dominance of a few highly-selected breeds. In response to this situation, there is a manifold of counter-movements in rural areas creating new food networks, partly based on the high quality production of pig and chicken meat. The nature of such newly emerging niche markets, and the meaning of “high quality production” within this scope, was elaborated on in chapter 1.1. For a number of these rural development initiatives, traditional pig and chicken breeds represent a cornerstone for their production and marketing strategies; therefore, niche markets may also be relevant for the maintenance of breed diversity. When traditional local breeds are compared with genotypes that are globally dominant, major differences in the growth rate of body tissues – one determinant of superior meat production efficiency of high yielding genotypes – are evident. The alterations in muscle microstructure and metabolism, which underlie the elevated growth rates of most conventional genotypes, could also have resulted in changes in the meat color and quality. In chapter 1.2, approaches investigating the effects that the genetic improvement of productivity and efficiency had on meat color and quality traits of pigs and chickens were introduced.

This thesis investigates two main areas: how far growth performance and the resulting genotypic differences could be related to a distinctive meat color and quality (papers I and III); and how this could be incorporated into production and marketing strategies (papers II and IV) in order to serve niche markets. Two contrasting approaches – the development of which is discussed in chapter 6.1 – toward these areas of research are used and outlined below.

In an on-station experiment, chicken lines differing in their growth performances were compared under standardized environmental conditions. The main issues pursued within paper I, chapter 2, are whether genotypic effects on chicken breast muscle color and quality exist, and how far these are dependent on slaughter age and weight. The results should contribute to the awareness of whether the application of slow-growing lines in chicken meat production systems potentially provides a distinctive meat color and quality. The dependence of meat color on physico-chemical meat traits and the variation in these relationships due to genotype and measuring parameters was evaluated in paper II, chapter 3. The findings of both papers are discussed in chapters 6.2 and 6.3.

The applied approach was embedded in a project aimed at the establishment of a short supply chain for pork from the native Vietnamese “Ban” pig breed in order to offer chances for rural smallholders in northwest Vietnam to diversify their incomes. The opportunities and constraints linking smallholder pig producers to urban niche markets for high quality pork products are elaborated on in chapter 6.4. Paper III, chapter 4, addresses the question of whether pork quality and color from a local Vietnamese pig breed, produced by marginalized smallholders in northern Vietnam, can be effectively discriminated from commercial pork from fatteners sired by a conventional genotype. These results should assist in positioning Ban products within the northern Vietnamese pork market and are discussed in chapter 6.4.1 and, particularly with respect to the color of pork, chapter 6.3. Additionally, in paper IV, chapter 5, options for the development of a marketing grid in order to

improve the economic profitability of the value chain for Ban pork, while considering the aspects of carcass and meat quality, are investigated. The relevance of the implementation of such a marketing grid on the livelihoods of the farmers is discussed in chapter 6.4.2.

## 1.4 References

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## **2 Paper I: Breast meat quality of chickens with divergent growth rates and its relation with growth curve parameters**

P.C. Muth, A. Valle Zárate

Animal Husbandry and Breeding in the Tropics and Subtropics, Hans-Ruthenberg-Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Garbenstr. 17, 70593 Stuttgart, Germany

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### **Rationale**

The effects of the increase of body weight of contemporary broilers during growth on functional meat quality and color characteristics of the chicken breast muscle are controversially debated at present.

### **Objective and scope**

- Male chickens (n=264) of a fast-growing commercial broiler and two slow-growing chicken lines were compared at equal age and at similar body weight in order to investigate the effects of growth rate on selected functional breast meat traits and meat color.
- Additionally, the breast meat characteristics of birds with different growth profiles were compared within lines.

### **Main Findings**

- When the body weight of commercial broilers reached to about 40 to 60% of their growth potential, they exhibited particularly high ultimate pH values compared with slow-growing lines.
- The ability of the meat of fast-growing broilers to retain water during cooking was impaired, which, in contrast to pH, was only marginally affected by body weight and/or age at slaughter.
- No adverse correlations of growth curve parameters derived from the Gompertz-Laird equation with breast meat functionality were detected within each of the investigated chicken lines. It is noteworthy that the associations of ultimate pH and cooking loss with maximum growth speed appear to follow a non-linear relationship.
- Thus, some of the functional characteristics of breast meat of the fast-growing broiler resembled the white striping defect described for poultry meat, but the hypothesis that selection on increased growth rates is detrimental for meat quality *per se* could not be confirmed. In fact, an elevated growth potential in particular, i.e. body weight at maturity, could have some beneficial effects for breast meat functionality, regardless of the genotypic growth rate.



## 2.1 Introduction

From the mid-20th century onwards, poultry breeders applied within-line selection schemes to improve production traits of meat-type chickens (later termed “broilers”) and maximize the profitability of chicken meat production. Hybridization allowed the achievement of further genetic improvement by additionally exploiting non-additive genetic effects, and, consequently, nowadays, broilers are the outcome of pyramidal-structured crossbreeding programmes, whereas in the top tier a few dozen purebred great-grandparental lines are selected for a broad range of traits (Neeteson-van Nieuwenhoven et al., 2013). Since the early days of poultry breeding, remarkable successes in economically important traits – such as growth rate, breast meat yield and feed efficiency – have been realized, as impressively demonstrated by Havenstein et al. (2003) and Schmidt et al. (2009), who compared growth performance and carcass composition of modern hybrids to lines that remained unselected since the 1950s.

In broiler production, the growth rate, given as body weight at a specific age, is of higher practical relevance than the mere growth potential, given as body weight at maturity, because slaughter generally takes place before the animals reach their mature body weight. It is thereby widely accepted that selection on muscle mass induces changes in both the number of prenatally formed myofibers and postnatal hypertrophy (Rehfeldt et al., 2004). Indeed, at day 18 of in ovo development, broiler embryos revealed a two-fold higher number of myofibers in the cross-section of breast muscles than layer chicks (Al-Musawi et al., 2011). Post hatch, at days 7 and 21 of age, both myofiber number and diameter increased for two broiler lines compared with a slow-growing layer (Scheuermann et al., 2004). By implication, comparing muscles at a given mass, slow-growing chickens must compensate for the reduced number of prenatally formed myofibers by increased hypertrophy. This was demonstrated by Rémignon et al. (1994) on a data set of 55 week old slow-growing chickens, which showed an increased muscle fiber size when compared to 11 week old fast-growing birds.

The effects of extensive modification in the development and histology of the skeletal muscle tissue of modern broiler chickens on product quality are controversially debated at present. In spite of the fact that genetic variation in growth traits of chickens is not exhausted yet, and thus allows for further selection (Neeteson-van Nieuwenhoven et al., 2013), it has been stressed that increasing the growth velocity and muscle mass in meat producing species may have reached their physiological limits, possibly resulting in trade-offs regarding product quality (Webb and Casey, 2010). In the case of fast-growing broilers, adverse responses towards selection on muscle mass development could be reflected in a compromised coping ability of supportive vascular and connective tissues, in altered enzymatic activities of skeletal muscle tissue, and in changes in the cation homeostasis (Dransfield and Sosnicki, 1999; Sandercock et al., 2006; Sandercock et al., 2009).

From a processors’ and costumers’ standpoint alterations in technological and sensorial properties of chicken meat in particular are of importance. Meat quality defects, which have been linked to selection on growth rate and breast muscle development in chickens, are, for instance, the pale, soft, exudative (PSE)-like and dark, firm, dry (DFD)-like meat conditions, white striping of breast meat, and “wooden” breast condition (Petracci et al., 2009; Kuttappan et al., 2012; Petracci et al., 2013; Kralik et al., 2014; Mudalal et al., 2015). These quality aberrations are associated with altered functional properties of meat, which, depending on the respective defect, can affect pH, color, water holding capacity, and tenderness of raw and/or cooked meat (Woelfel et al., 2002; Wilhelm et al., 2010; Petracci et al., 2013; Mudalal et al., 2015). However, it was also suggested that, although intense

selection on growth performance and breast yield elicited differences in muscle tissue histology and metabolism, there is little evidence of adverse effects on meat quality in chickens besides slight modifications in color (Rémignon and Le Bihan Duval, 2003; Duclos et al., 2007).

Public concerns about animal health and welfare issues in the broiler industry and customer preferences for “terroir products” promoted the continued or resurgent use of slow-growing lines in chicken meat production. Given the ambition of further elevating the growth performance of modern broilers to respond to the increasing global demand for chicken meat on the one hand, and differentiation towards slow-growing strains on the other, it is important to know in which breast meat quality traits chicken lines varying in growth rates differ.

In this study, chicken lines strongly divergent in growth rate were compared under standardized conditions in order to investigate the effects of genotypic determined growth rate on functional meat quality and color characteristics of breast fillets. The subject was approached from the following perspectives: (1) comparing fast-growing and slow-growing chicken lines at equal age and at similar body weight (thus, different slaughter ages were applied); and (2) comparing individual chickens with different growth trajectories within fast-growing and slow-growing lines.

## **2.2 Material and methods**

### **Animals, rearing and slaughter**

The experimental protocol was approved by the Animal Policy and Welfare Commissioner of the University of Hohenheim (Stuttgart, Germany). In total, 264 male birds of three lines were used in the periods from May until July 2012 and from June until August 2013. The slow-growing lines were represented by two experimental meat-type chicken lines kept by the University of Hohenheim and moderately selected for growth rate since 2001. One experimental line was established by crossing the Rhode Island breed with the Silky fowl; this line carried the sex-linked recessive inhibitor of dermal melanin and dominant fibromelanosis genes, which, in combination, cause hyperpigmentation of skin and skeletal muscle tissue. Thus, this line was characterized by a slow growth rate and its dark skin and meat color (slow-growing, dark-skinned: SGD). The second experimental line was established by crossing the Rhode Island breed with the New Hampshire breed. This line was also slow-growing, but exhibited a light (i.e. normal) skin and meat color (slow-growing, light-skinned: SGL). Commercial broilers (Ross 308, Aviagen Group, Huntsville, AL, USA) characterized by a fast growth rate and a light skin color (fast-growing, light-skinned: FGL) were obtained from a commercial hatchery (Brüterei Süd of the Weser-Ems GmbH & Co. KG, Regenstauf, Germany). Birds from each line were distributed to each of two indoor pens. All birds were individually identified with numbered tags and kept on wood shavings at a density of ~7 birds/m<sup>2</sup> with ad libitum access to feed and water. Lighting was provided for 24 h for the first 48 h post hatch and subsequently for 18 h per day until the end of the experiment. With respect to feeding it was attempted to provide standardized, non-limiting conditions. A starter diet was fed from 0-3 weeks of age and a grower diet from 3-10 weeks of age (Table 2.1). Body weight was recorded weekly for each bird. Commercial broilers were slaughtered at 4, 7 and 10 weeks of age, and birds of the experimental lines were slaughtered at 7 and 10 weeks of age. Feed was withdrawn approximately 12 h before slaughter. After electrical stunning (110 mA), the neck was cut and the birds bled. Carcasses were scalded in a 65°C water bath for 20 s and defeathered in a rotary drum picker. Head and neck as well as feet and shanks were removed. Carcasses were eviscerated manually. After chilling for 2 h ( $\pm$ 1 h) at 4°C, the breast muscle (*Pectoralis major* and *minor*) was removed and

weighed without skin. The left *Pectoralis major* muscle was stored refrigerated at 4°C and used for the determination of meat quality parameters. From the right *Pectoralis major* muscle, 15 g slices were harvested at a medial position and stored in plastic tubes at -20°C for pigment analysis.

**Table 2.1** Composition and main characteristics of diets

	Diet	
	Starter (week 0-3) <sup>1</sup>	Grower (week 3-10) <sup>2</sup>
<b>Composition (g/kg)</b>		
Soybean meal	265	234
Corn	250	—
Wheat	208	692
Triticale	100	—
Soybean, whole seed	70	—
Vegetable oil <sup>3</sup>	20	—
Soybean oil	13	33
Hydrothermally processed wheat	15	—
Hydrothermally processed corn	7.5	—
Hydrothermally processed barley	7.5	—
CaCO <sub>3</sub>	15	17
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	13	17
NaHCO <sub>3</sub>	2.4	3.0
NaCl	1.8	3.0
Ca(C <sub>2</sub> H <sub>5</sub> COO) <sub>2</sub>	—	2.0
<b>Nutrient concentrations (calculated)</b>		
Metabolisable energy (MJ/kg)	12.6	12.5
Crude protein (g/kg)	212.0	194.1
Lysine (g/kg)	14.0	9.2
Methionine (g/kg)	6.5	3.4
Crude lipids (g/kg)	67.0	51.3
Crude fibre (g/kg)	29.0	31.1
Crude ash (g/kg)	62.0	52.1
Ca (g/kg)	9.0	7.6
P (g/kg)	7.0	4.6
Na (g/kg)	1.4	1.4

<sup>1</sup>Premix added to the starter provided the following per kilogram of diet: vitamin A, 15,500 IU; vitamin D3, 5,000 IU; vitamin E (DL- $\alpha$ -tocopheryl acetate), 70 mg; Mn, 80 mg; Fe, 60 mg; Zn, 50 mg; Cu, 17 mg; I, 1.01 mg; Se, 0.44 mg; Co, 0.38 mg; 3-phytase, 600 FTU; endo-1,4- $\beta$ -Xylanase, 12 IU; Nasarin (coccidiostat), 48 mg; Nicarbazin, 48 mg (coccidiostat).

<sup>2</sup>Premix added to the grower provided the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 40 mg; vitamin K, 3 mg; thiamine, 3 mg; riboflavin, 6 mg; cobalamin, 30  $\mu$ g; niacin, 50 mg; pantothenic acid, 12 mg; folic acid, 1,000  $\mu$ g; biotin, 100  $\mu$ g; Mn, 108 mg; Fe, 80 mg; Zn, 72 mg; Cu, 14 mg; I, 1.44 mg; Se, 0.45 mg.

<sup>3</sup>Soy oil, palm oil, sunflower oil, rapeseed oil, coconut oil.

## Meat quality analysis

At 24 h post-mortem, pH of the left breast muscle was recorded with a pH meter equipped with a glass electrode, which was calibrated prior to use at pH 4.0 and 7.0. Duplicate measurements at 1 cm depth on the medial portion of each breast fillet were averaged.

The L\*a\*b\* color profile (L\* = lightness, a\* = redness, b\* = yellowness) according to the Commission Internationale d'Éclairage was determined on the cranial portion of the dorsal (adjacent to the bone) surface of the left breast fillet at 24 h post-mortem. From the a\* and b\* values the color saturation (chroma,  $C^* = (a^{*2} + b^{*2})^{0.5}$ ) and the hue angle ( $h^\circ = \tan^{-1}(b^*/a^*) \cdot 180/\pi$ ) were derived. A chromameter (CR-400, Konica Minolta Sensing Inc., Tokyo, Japan) with an illuminant C and 2° observer and 8 mm aperture size setting was used and calibrated prior to each use with a white ceramic tile. For each muscle, four readings were averaged.

The expressible moisture of breast meat was determined at 24 h post-mortem through the filter paper press method according to van Oeckel et al. (1999). Approximately 0.3 g of meat was removed from the left breast fillet, placed on filter paper, and pressed between two parallel blocks for 5 min. The areas of pressed meat and expressed moisture were measured using a planimeter, and the ratio of meat to the wet area on the paper (M/W, in  $\text{cm}^2/\text{cm}^2$ ) was multiplied by 100. Values close to 100 and 0 indicate a high and low water holding capacity, respectively.

For the determination of cooking loss at 24 h post-mortem, a sample was cut perpendicular to the fibre orientation from the left breast. The thickness of the samples was standardized to approximately 2 cm. The samples were then placed in a plastic bag and immersed in boiling water with the sealed bag opening extending above the water surface (Petracchi and Baéza, 2011). The samples were cooked to an internal temperature of 85°C, whereas the temperature was controlled using a digital insertion thermometer. After cooling the samples to room temperature, moisture was blotted, samples were weighed and the cooking loss expressed as weight loss during cooking relative to the initial weight of the sample.

## Pigment determination

The extraction of hemin (He, in ppm) was carried out in duplicate, according to Hornsey's (1956) method with slight modifications. Slices ( $5 \pm 0.02$  g) of the right breast muscle were minced and mixed with 20 ml of 80% acetone solution and 0.5 ml of HCl. Water was added until total water in the mixture equalled 4.5 g. The mixture was homogenized, held for at least 1 h at subdued light, and then centrifuged twice. The absorbance of the centrifugate was read at 640 and 730 nm. The hemin content of the extract was calculated as follows:

$$\text{He (ppm)} = (A_{640} - A_{730}) / 4.8 \times 652 \times \text{dilution factor},$$

where  $A_{640}$  = absorbance at a wavelength of 640 nm;  $A_{730}$  = absorbance at a wavelength of 730 nm; 4.8 = millimolar extinction coefficient  $\epsilon_\lambda/\text{mM}/\text{cm}$  at 640 nm (Hornsey, 1956); 652 = molecular mass of hemin in Da (Hornsey, 1956).

## Statistical analysis

To assure sufficient numbers of observation in spite of limited testing facilities, the experiment was reproduced as a whole in two consecutive years (blocks), using the same experimental protocol, personnel, facilities and equipment. The statistical analysis was conducted by R software version 3.0.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). The chicken line and the age at slaughter were combined in one treatment effect (line-age). The individual bird represented one experimental unit. The number of observations for each factor level combination can be derived from Table 2.2. For the evaluation of the effect of treatment on body weight, carcass characteristics and breast meat quality, the data were analyzed in a full factorial (7 x 2) experimental design. A general linear model with fixed factors for treatment (seven levels), year (two levels), and their interaction was fitted for each response. If necessary, a log-transformation was applied. For the hemin concentration, a linear mixed model including a random effect accounting for the day of laboratory analysis was applied. For the color traits, only the light-skinned lines were considered. For the evaluation of ultimate pH values nine observations exceeding a pH value of 6.0 had to be removed, because otherwise the assumption of normally distributed residuals was violated. For the evaluation of hue angle 14 observations had to be removed, because they exhibited b\* values lower than zero. Least squares means for treatments were compared pairwise using the adjustment of P-values according to the Tukey method for unbalanced data. Differences between treatments with  $P < 0.05$  were considered significant.

**Table 2.2** Number of birds for each combination of chicken line, slaughter age and year

Line-age <sup>1</sup>		2012	2013	Total
SGL -	7 wk	20	20	40
	10 wk	20	20	40
SGD -	7 wk	20	20	40
	10 wk	20	20	40
FGL -	4 wk	20	25	45
	7 wk	15	15	30
	10 wk	15	14	29

<sup>1</sup>Combination of chicken line (SGL = slow-growing light-skinned; SGD = slow-growing dark-skinned; FGL = fast-growing light-skinned) and slaughter age.

For the analysis of the association of the growth trajectory with meat quality traits, the data set was limited to birds slaughtered at 7 and 10 weeks of age. The Gompertz-Laird function (1) was fitted to the weekly body weight records of each individual bird by non-linear regression, and the asymptotic body weight (2) and point of inflexion (3), as well as the weight gain at inflexion (4), were calculated (Koncagul and Cadirci, 2009):

$$BW_{wk} = BW_0 * e^{((L/K)(1-e^{-Kwk}))} \quad (1),$$

$$BW_A = BW_0 * e^{(L/K)} \quad (2),$$

$$POI = 1/K * \ln(L/K) \quad (3),$$

$$DG_{max} = K * BW_{POI} \ln(BW_A / BW_{POI}) \quad (4),$$

where  $BW_{wk}$  is the body weight of birds at week  $wk$ ,  $BW_0$  is the initial weight,  $L$  is the initial specific growth rate,  $K$  is the exponential decay of the initial specific growth rate,  $BW_A$  is the asymptotic body weight,  $POI$  is the point of inflexion,  $DG_{max}$  is maximum daily gain, that is, the weight gain at inflexion, and  $BW_{POI}$  is the body weight at inflexion ( $BW_A$  multiplied by 0.368).

The essential growth curve parameters ( $BW_A$ ,  $POI$ ,  $DG_{max}$ ) were compared amongst treatments by adapting the general linear models described before.

The relationships between the growth curve parameters and meat quality and color traits were evaluated for each line separately, controlling for the effects of age (7 and 10 weeks) and year by performing partial correlation of the responses for each pair of growth curve parameters and meat quality traits.

### 2.3 Results

As expected, line and slaughter age significantly contributed to variation in growth performance and carcass quality traits (Table 2.3), but most parameters were also affected by year and the interaction between line-age and year. The superior growth rate of FGL birds was evidenced in both years by significantly ( $P<0.05$ ) higher body weights at equal slaughter age. At 4 weeks of age the body weight of broilers was similar or lower compared to 10 week old slow-growing birds. At equal slaughter age, commercial broilers exhibited substantially increased dressing percentages compared to both slow-growing lines ( $P<0.05$ ). In the first year, the breast weights from commercial broilers were significantly increased compared to those of slow-growing birds ( $P<0.05$ ), irrespective of the age and body weight at slaughter. However, in the second year, the weight of the breast from 4 week old FGL broilers was similar to the breast weights of both slow-growing lines slaughtered at an age of 10 weeks ( $P>0.05$ ). The breast yields of the slow-growing lines were still significantly ( $P<0.05$ ) lower than those of 4 week old broilers for both years of the experiment.

**Table 2.3** Effect of the combination of chicken line, slaughter age and year on body weight and carcass composition

Line-age <sup>1</sup>	Weight at slaughter <sup>2</sup> , g		Dressing percentage <sup>3</sup> , %		Breast weight <sup>2</sup> , g		Breast yield <sup>2</sup> , %	
	2012	2013	2012	2013	2012	2013	2012	2013
SGL - 7 wk	1202 <sup>e</sup>	1164 <sup>e</sup>	63.1±0.31 <sup>d</sup>	64.3±0.31 <sup>d</sup>	124 <sup>f</sup>	111 <sup>e</sup>	10.3 <sup>cd</sup>	9.8 <sup>e</sup>
10 wk	1761 <sup>cx</sup>	1984 <sup>cy</sup>	65.6±0.31 <sup>c</sup>	66.8±0.31 <sup>c</sup>	198 <sup>d</sup>	215 <sup>c</sup>	11.2 <sup>c</sup>	10.8 <sup>d</sup>
SGD - 7 wk	1167 <sup>ex</sup>	1006 <sup>fy</sup>	63.1±0.31 <sup>d</sup>	63.4±0.31 <sup>d</sup>	116 <sup>fx</sup>	92 <sup>fy</sup>	9.9 <sup>d</sup>	9.2 <sup>e</sup>
10 wk	1468 <sup>dy</sup>	1624 <sup>dx</sup>	63.6±0.31 <sup>dy</sup>	66.6±0.31 <sup>cx</sup>	146 <sup>ey</sup>	178 <sup>dx</sup>	9.9 <sup>dy</sup>	11.0 <sup>dx</sup>
FGL - 4 wk	1423 <sup>dx</sup>	1245 <sup>ey</sup>	65.5±0.31 <sup>cy</sup>	67.2±0.28 <sup>cx</sup>	261 <sup>cx</sup>	204 <sup>cdy</sup>	18.4 <sup>bx</sup>	16.4 <sup>cy</sup>
7 wk	3082 <sup>b</sup>	2880 <sup>b</sup>	72.2±0.36 <sup>b</sup>	72.1±0.36 <sup>b</sup>	604 <sup>b</sup>	526 <sup>b</sup>	19.6 <sup>ab</sup>	18.0 <sup>b</sup>
10 wk	4668 <sup>ab</sup>	4450 <sup>a</sup>	74.7±0.36 <sup>a</sup>	75.3±0.37 <sup>a</sup>	991 <sup>a</sup>	892 <sup>ab</sup>	21.2 <sup>a</sup>	20.1 <sup>a</sup>
<b>Level of significance</b>								
<b>Line-age<sup>1</sup></b>	<0.001		<0.001		<0.001		<0.001	
<b>Year</b>	0.012		<0.001		<0.001		<0.001	
<b>Line-age<sup>1</sup> x year</b>	<0.001		<0.001		<0.001		<0.001	

a-e Within a column, different letters indicate significant differences between treatments ( $P \leq 0.05$ ).

x,y Within a row, different letters indicate significant differences between years ( $P \leq 0.05$ ).

<sup>1</sup>Combination of chicken line (SGL = slow-growing light-skinned; SGD = slow-growing dark-skinned; FGL = fast-growing light-skinned) and slaughter age.

<sup>2</sup>Back-transformed values are presented; pairwise comparisons were based on the log-transformed response.

<sup>3</sup>Data presented as least squares means  $\pm$  standard error.

Seven and 10 week old FGL broilers exhibited significantly higher ultimate pH values (pH measured at 24 h post-mortem) than 4 week old broilers ( $P < 0.05$ ; Table 2.4). Notably, most of the observations that exhibited pH values above pH 6.0 and had to be excluded from the statistical analysis were older broiler chickens, corroborating the results presented. The slow-growing lines did not differ significantly from each other ( $P > 0.05$ ) and showed ultimate pH values in-between the broilers slaughtered at an age of 7 and 10 weeks and the younger ones. The cooking loss of breast meat was significantly higher for FGL chickens compared with both slow-growing lines ( $P < 0.05$ ), irrespective of the slaughter age and year. Additionally, significant differences in cooking loss were detected among slow-growing chickens in the first year of the experiment ( $P < 0.05$ ), whereas in the second year no differences among slow-growing birds were observed ( $P > 0.05$ ). Cooking losses were slightly, but significantly, higher for 10 week old FGL broilers compared to 4 week old FGL birds in the second year of the experiment ( $P < 0.05$ ); however, this finding was not corroborated by the first year. Notably, cooking losses of the FGL birds were significantly increased in the second year compared to the first year ( $P < 0.05$ ). According to the filter paper press method, breast meat from FGL birds slaughtered at an age of 7 weeks exhibited a compromised water holding capacity. The area of pressed meat relative to the area of expressible moisture (M/W values) of 7 week old broilers was significantly reduced compared to SGD chickens in the first year of the experiment ( $P < 0.05$ ). This result was reproduced in the second year for most of the age groups of the slow growing lines. The differences in heme pigment concentrations were marginal among the line-age combinations. Only when pooling data for both experimental years, significantly higher heme contents for SGD birds compared with commercial broilers at an age of 7 weeks were detected ( $P < 0.05$ ).



**Table 2.4** Effect of the combination of chicken line, slaughter age and year on the ultimate pH, water holding capacity and pigment concentration of breast meat

Line-age <sup>1</sup>	Ultimate pH		Cooking loss, %		M/W <sup>2</sup> , cm <sup>2</sup> /cm <sup>2</sup>		Hemin, ppm	
	2012	2013	2012	2013	2012	2013	2012	2013
SGL - 7 wk	5.67±0.02 <sup>cdy</sup>	5.76±0.02 <sup>abcx</sup>	17.0±0.7 <sup>bc</sup>	15.4±0.7 <sup>c</sup>	50.9±1.6 <sup>abc</sup>	54.1±1.7 <sup>a</sup>	8.84±0.55	11.66±0.58
10 wk	5.66±0.02 <sup>cdy</sup>	5.74±0.02 <sup>abcx</sup>	13.4±0.7 <sup>d</sup>	16.5±0.7 <sup>c</sup>	49.9±1.7 <sup>abc</sup>	53.2±1.6 <sup>a</sup>	10.03±0.55	9.55±0.58
SGD - 7 wk	5.69±0.02 <sup>bcd</sup>	5.73±0.02 <sup>bc</sup>	18.5±0.7 <sup>b</sup>	15.8±0.7 <sup>c</sup>	57.5±1.9 <sup>a</sup>	53.3±1.7 <sup>a</sup>	9.58±0.55	13.06±0.61
10 wk	5.71±0.02 <sup>bc</sup>	5.73±0.02 <sup>bc</sup>	14.8±0.7 <sup>cd</sup>	16.1±0.7 <sup>c</sup>	54.6±1.7 <sup>ab</sup>	51.5±1.7 <sup>ab</sup>	9.12±0.55	9.53±0.56
FGL - 4 wk	5.63±0.02 <sup>d</sup>	5.69±0.01 <sup>c</sup>	23.1±0.7 <sup>ay</sup>	26.6±0.6 <sup>bx</sup>	48.8±1.7 <sup>bc</sup>	47.9±1.5 <sup>ab</sup>	11.46±0.58	10.60±0.51
7 wk	5.79±0.02 <sup>a</sup>	5.79±0.02 <sup>ab</sup>	23.2±0.8 <sup>ay</sup>	28.4±0.8 <sup>abx</sup>	45.2±1.9 <sup>c</sup>	44.2±1.9 <sup>b</sup>	8.21±0.63	10.14±0.63
10 wk	5.77±0.02 <sup>ab</sup>	5.83±0.02 <sup>a</sup>	22.5±0.8 <sup>ay</sup>	31.3±0.8 <sup>ax</sup>	47.5±2.0 <sup>bc</sup>	51.7±1.9 <sup>ab</sup>	8.76±0.68	10.29±0.66
<b>Level of significance<sup>3</sup></b>								
<b>Line-age<sup>1</sup></b>	<0.001		<0.001		<0.001		0.015	
<b>Year</b>	<0.001		<0.001		0.833		0.055	
<b>Line-age<sup>1</sup> x year</b>	0.129		<0.001		0.100		0.107	

Data presented as least squares means ± standard error.

a-d Within a column, different letters indicate significant differences between treatments (P≤0.05).

x,y Within a row, different letters indicate significant differences between years (P≤0.05).

<sup>1</sup>Combination of chicken line (SGL = slow-growing light-skinned; SGD = slow-growing dark-skinned; FGL = fast-growing light-skinned) and slaughter age.

<sup>2</sup>M/W = ratio of meat area relative to the wet area multiplied by 100 after applying the filter paper press method.

<sup>3</sup>Level of significance for hemin concentration calculated according to Wald chi-square test.

The color profiles determined at 24 h post-mortem on the dorsal surface of breast fillets of the light-skinned lines revealed significant differences (Table 2.5). The L\* values of breast meat from 4 week old FGL broilers were significantly increased compared to SGL chickens ( $P < 0.05$ ), whereas at equal age the L\* values of breast meat did not differ among lines ( $P > 0.05$ ). Despite the small effect size, the differences in the lightness of breast meat were reproducible. On the other hand, the slight but significant differences in the redness of breast meat among treatments noted in the first year ( $P < 0.05$ ) were not observed in the second year. This might be due to the low level of redness in meat of the broilers in the second year. While at an equal age both lines did not differ in the yellowness of breast meat ( $P > 0.05$ ), the b\* values were significantly increased for younger birds in the first year of the experiment ( $P < 0.05$ ). In the second year, this finding could be substantiated only for the broilers. The differences in the a\* and b\* components were reflected in the polar color coordinates. The color saturation of breast meat from birds slaughtered at an equal age did not differ ( $P > 0.05$ ). However, the meat color of 4 week old broilers was more intense compared to most other line-age groups ( $P < 0.05$ ). The hue angle was significantly increased for 4 week old broilers compared to 10 week old birds of both lines in the first year ( $P < 0.05$ ), and compared to all other line-age groups in the second year of the experiment ( $P < 0.05$ ). The hue angle did not differ among chickens slaughtered at equal age ( $P > 0.05$ ). For the analysis of hue angle particular observations, namely those with b\* values below zero, had to be excluded from the statistical analysis, possibly limiting the solidity of the statements.

**Table 2.5** Effect of the combination of chicken line, slaughter age and year on the color of breast meat

Line-age <sup>1</sup>	Lightness, L*		Redness, a*		Yellowness, b*		Chroma, C*		Hue, h°		
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	
SGL -	7 wk	48.7±0.5 <sup>b</sup>	47.8±0.5 <sup>bc</sup>	3.88±0.21 <sup>c</sup>	3.85±0.21	2.10±0.20 <sup>bx</sup>	1.03±0.20 <sup>by</sup>	4.49±0.21 <sup>bc</sup>	4.06±0.21 <sup>b</sup>	28.4±2.1 <sup>ax</sup>	17.0±2.2 <sup>by</sup>
	10 wk	49.1±0.5 <sup>b</sup>	47.2±0.5 <sup>c</sup>	4.16±0.21 <sup>bc</sup>	4.06±0.21	0.78±0.20 <sup>c</sup>	1.00±0.20 <sup>b</sup>	4.31±0.21 <sup>c</sup>	4.24±0.21 <sup>b</sup>	13.8±2.3 <sup>b</sup>	14.9±2.2 <sup>b</sup>
FGL -	4 wk	51.4±0.5 <sup>a</sup>	50.4±0.4 <sup>a</sup>	5.52±0.21 <sup>a</sup>	4.22±0.19	3.21±0.20 <sup>a</sup>	3.27±0.18 <sup>a</sup>	6.49±0.21 <sup>ax</sup>	5.39±0.19 <sup>ay</sup>	30.4±2.1 <sup>a</sup>	38.1±1.9 <sup>a</sup>
	7 wk	50.4±0.5 <sup>ab</sup>	49.8±0.5 <sup>ab</sup>	4.96±0.25 <sup>abx</sup>	3.48±0.25 <sup>y</sup>	2.02±0.23 <sup>b</sup>	1.25±0.23 <sup>b</sup>	5.45±0.25 <sup>abx</sup>	3.80±0.25 <sup>by</sup>	22.9±2.5 <sup>ab</sup>	22.8±2.6 <sup>b</sup>
	10 wk	49.8±0.5 <sup>ab</sup>	48.1±0.6 <sup>bc</sup>	4.99±0.25 <sup>abx</sup>	3.53±0.25 <sup>y</sup>	0.83±0.23 <sup>c</sup>	0.47±0.24 <sup>b</sup>	5.11±0.25 <sup>bcx</sup>	3.65±0.26 <sup>by</sup>	11.4±2.6 <sup>b</sup>	14.1±3.0 <sup>b</sup>
<b>Level of significance</b>											
Line-age <sup>1</sup>	<0.001		<0.001		<0.001		<0.001		<0.001		
Year	<0.001		<0.001		0.008		<0.001		0.988		
Line-age <sup>1</sup> x year	0.636		<0.001		0.008		0.002		<0.001		

Data presented as least squares means ± standard error.

a-d Within a column, different letters indicate significant differences between treatments (P≤0.05).

x,y Within a row, different letters indicate significant differences between years (P≤0.05).

<sup>1</sup>Combination of chicken line (SGL = slow-growing light-skinned; FGL = fast-growing light-skinned) and slaughter age.

In regard to the growth profile (Table 2.6), the estimates for  $BW_A$  were significantly increased for commercial broilers compared with both slow-growing lines at an equal slaughter age ( $P < 0.05$ ). At a slaughter age of 10 weeks, the SGL chickens showed a significantly increased asymptotic body weight compared to SGD birds ( $P < 0.05$ ). In the first year, the POI was significantly greater for broilers slaughtered at an age of 10 weeks compared to most other line-age combinations ( $P < 0.05$ ), whereas in the second year the estimated POI did not differ among lines at an equal age ( $P > 0.05$ ). The estimation for  $DG_{max}$  was significantly greater for broilers compared with both slow-growing lines ( $P < 0.05$ ), irrespective of the age at slaughter and year. In both years, there was a significant difference in the estimated  $DG_{max}$  between the two slow growing lines. Within the lines, significant partial correlations between growth curve parameters and functional meat properties were detected (Table 2.7). For broilers,  $BW_A$  was significantly negatively associated with cooking loss ( $P < 0.05$ ) and positively with M/W values ( $P < 0.05$ ). Body weight at maturity was also negatively correlated to cooking loss for SGL birds ( $P < 0.05$ ). A delayed POI correlated significantly with reduced cooking losses ( $P < 0.05$ ) and increased  $a^*$  values ( $r = 0.25$ ;  $P < 0.05$ ) of the breast meat from SGL chickens. The  $DG_{max}$  was significantly positively related to ultimate pH ( $P < 0.05$ ) and negatively to the lightness ( $r = -0.29$ ;  $P < 0.05$ ) of breast meat from broilers. Interestingly, cooking loss was significantly negatively correlated to  $DG_{max}$  in both slow-growing lines ( $P < 0.05$ ), but not within the fast-growing broilers.

**Table 2.6** Effect of the combination of chicken line, slaughter age and year on Gompertz-Laird growth curve parameters

Line-age <sup>1</sup>	Body weight (asymptotic) <sup>2</sup> , g		Point of inflexion <sup>3</sup> , d		Maximum daily gain <sup>2</sup> , g	
	2012	2013	2012	2013	2012	2013
SGL - 7 wk	2059 <sup>cd</sup>	1889 <sup>d</sup>	32.6±1.1 <sup>b</sup>	32.6±1.1 <sup>b</sup>	33.6 <sup>b</sup>	30.6 <sup>d</sup>
10 wk	2328 <sup>cy</sup>	3855 <sup>bx</sup>	36.5±1.1 <sup>aby</sup>	54.1±1.1 <sup>ax</sup>	32.9 <sup>by</sup>	39.0 <sup>cx</sup>
SGD - 7 wk	1969 <sup>cd</sup>	1652 <sup>d</sup>	32.2±1.1 <sup>b</sup>	33.0±1.1 <sup>b</sup>	32.9 <sup>bx</sup>	27.0 <sup>ey</sup>
10 wk	1771 <sup>dy</sup>	3130 <sup>cx</sup>	32.9±1.1 <sup>by</sup>	53.8±1.1 <sup>ax</sup>	26.9 <sup>cy</sup>	31.3 <sup>dx</sup>
FGL - 7 wk	5355 <sup>b</sup>	4545 <sup>b</sup>	34.1±1.3 <sup>b</sup>	32.6±1.3 <sup>b</sup>	84.2 <sup>a</sup>	77.4 <sup>b</sup>
10 wk	6917 <sup>ay</sup>	8779 <sup>ax</sup>	41.7±1.3 <sup>ay</sup>	53.3±1.5 <sup>ax</sup>	87.4 <sup>a</sup>	90.2 <sup>a</sup>
	<b>Level of significance</b>					
Line-age <sup>1</sup>	<0.001		<0.001		<0.001	
Year	<0.001		<0.001		0.942	
Line-age <sup>1</sup> x year	<0.001		<0.001		<0.001	

a-e Within a column, different letters indicate significant differences between treatments (P≤0.05).

x,y Within a row, different letters indicate significant differences between years (P≤0.05).

<sup>1</sup>Combination of chicken line (SGL = slow-growing light-skinned; SGD = slow-growing dark-skinned; FGL = fast-growing light-skinned) and slaughter age.

<sup>2</sup>Back-transformed values are presented; pairwise comparisons were based on the log-transformed response.

<sup>3</sup>Data presented as least squares means ± standard error.

**Table 2.7** Residual Pearson product-moment correlation coefficients between Gompertz-Laird growth curve parameters and breast meat quality traits by line

Line <sup>1</sup>	Growth parameter <sup>2</sup>	Trait <sup>3</sup>			
		pHu	CL	M/W	He
SGL					
	BW <sub>A</sub>	0.12	-0.26*	0.00	-0.08
	POI	0.16	-0.24*	-0.07	-0.10
	DG <sub>max</sub>	0.16	-0.24*	0.22*	0.03
SGD					
	BW <sub>A</sub>	0.06	-0.11	0.23*	-0.05
	POI	0.17	0.01	0.11	0.09
	DG <sub>max</sub>	-0.16	-0.36**	-0.06	0.06
FGL					
	BW <sub>A</sub>	0.21	-0.32*	0.28*	0.19
	POI	0.16	-0.05	0.20	0.17
	DG <sub>max</sub>	0.29*	-0.06	0.03	0.14

Pearson product moment correlation coefficients significantly different from zero are represented with \* at P<0.05 and \*\* at P<0.01.

<sup>1</sup>SGL = slow-growing light-skinned; SGD = slow-growing dark-skinned; FGL = fast-growing light-skinned.

<sup>2</sup>BW<sub>A</sub> = asymptotic body weight; POI = point of inflexion; DG<sub>max</sub> = maximum daily gain.

<sup>3</sup>pHu = ultimate pH; CL = cooking loss; M/W = ratio of meat area relative to the wet area after applying the filter paper press method; He = hemin concentration.

## 2.4 Discussion

In the present experiment, the effects of growth rate and growth potential on breast meat quality and color of chickens were investigated. The experimental design allowed partly accounting for confounding variables (age and body weight) by applying an additional (lower) slaughter age to broilers. Studies on the effect of selecting for growth performance on meat quality of poultry species, which have been conducted before, were either based on comparison at similar live weight (e.g., N'dri et al., 2007), which corresponds well to practical scenarios, but implies that the age of slow-growing chickens at slaughter is markedly increased, or at an equal chronological age of chicken strains (e.g., Berri et al., 2001; Lonergan et al., 2003; Sandercock et al., 2009) and turkey lines (Fernandez et al., 2001; Werner et al., 2008), accepting a wide range in body weight.

The broiler exhibited average daily gains common for modern broilers, whereas the average daily gains of the slow-growing experimental chicken lines were slightly higher compared to traditional and layer strains, but lower compared to most slow-growing commercial broilers. Thus, the broilers could exploit their genetic potential for growth performance and carcass quality. In spite of large variability in growth and carcass traits among individuals at low numbers of observations, the broilers clearly outperformed the slow-growing lines and attained significantly increased body weights at an equal slaughter age. The superior growth performance of FGL birds compared to both slow-growing lines was also reflected in the Gompertz-Laird growth curve parameters, particularly in significantly increased maximum daily gains of the broilers. Also the breast muscle yield of FGL was markedly increased compared to the slow-growing lines.

### **Effects of line and slaughter age on the ultimate pH of breast meat**

In the present study, differences in ultimate pH between fast- and slow-growing lines depended on the confounded effects of age and/or body and breast muscle weight. Thereby, broilers at an age of 4 weeks revealed lower ultimate pH values than broilers slaughtered at 7 and 10 weeks, the slow-growing lines being intermediate. In agreement with the results of the present study, Baéza et al. (2012) reported that the ultimate pH of commercial broilers increased with slaughter age. Berri et al. (2001) found an increased ultimate pH for the breast muscle of a heavier fast-growing line (commercial selected line) compared with their lighter unselected counterparts (commercial control line). Ultimate pH values for broilers were significantly increased compared to slow-growing chickens when compared at similar body weight (approximately 2 kg) (N'dri et al., 2007), and for broilers compared to Leghorn chickens at both equal age and weight (approximately 1.5 kg) (An et al., 2010). No genotype effect on pH values was found comparing slow- and fast-growing chicken strains at the same chronological age by Lonergan et al. (2003). Finally, ultimate pH was even elevated for slow-growing lines compared with broiler strains slaughtered at 8 weeks of age (Sandercock et al., 2009), and for a medium-growing line compared with a fast-growing strain slaughtered at 81 days of age (Sirri et al., 2011). Berri et al. (2007) detected a positive genetic relationship between myofiber diameter and ultimate pH, which is possibly rather related to the reduced glycolytic potential of larger myofibers than to an altered enzymatic activity (Berri et al., 2001). In another study, breast muscle weight was genetically positively correlated with ultimate pH (Le Bihan-Duval et al., 2008). These studies suggest that variation in the size of myofibers in the breast muscle may account for the differences in ultimate pH detected. Contradictory results between studies can possibly be explained by the relation of the age at slaughter to the age when radial growth of myofibers is accelerated (i.e. age at POI). When slaughtered at an age of 4 weeks, the FGL chickens had just attained about 17% of their mature body weight and had not reached the POI yet, but at an age of 7 and 10 weeks, they had passed this point. In fact, ultimate pH was also positively correlated with  $DG_{max}$  in FGL chickens, indicating that increasing the maximum growth rate through radial and longitudinal enlargement of myofibers reduces the extent of acidification of breast meat. In contrast, ultimate pH was not associated with maximum growth speed in both slow-growing lines, indicating that ultimate pH and peak growth rate follow a non-linear relationship.

### **Effects of line and slaughter age on water holding capacity of breast meat**

The ability of meat to retain water during storage and cooking is an important functional meat quality attribute, with implications for the processing ability and eating experience of chicken meat. Irrespective of the age at slaughter, fast-growing chickens displayed markedly reduced cooking yields. In contrast, the water holding capacity of raw meat was somewhat reduced for broilers slaughtered at an age of 7 weeks, but acceptable for FGL chickens slaughtered at 4 and 10 weeks of age. No differences in drip loss at days three and six of storage between selected and unselected chicken lines (Berri et al., 2001) or in drip and cook loss between a medium- and a fast-growing strain (Sirri et al., 2011) were found. According to Lonergan et al. (2003), cooking losses did not differ when comparing broilers and their crosses with layers and traditional chickens at equal age, but were significantly increased for the respective purebred slow-growing lines. It is notable that within slow-growing birds high maximum daily gain rates were positively correlated to cooking yields, which was not applicable for the broilers. But, the growth potential of the broilers, given as  $BW_A$ , had a favorable association with cooking loss and the expressible moisture of breast meat, indicating that even in fast-growing strains, simultaneous improvements in water retention and growth

performance are not precluded, but can be achieved by increasing the growth potential rather than the growth speed. Gaya et al. (2011) reported a moderate and negative genetic correlation between breast weight and thawing-cooking loss for a commercial sire line, which is similar to our results. The genotypic differences in breast meat functionality, particularly those between fast- and slow-growing lines, are nevertheless not straightforwardly interpretable. A rapid pH fall and high temperatures during the early post-mortem phase can trigger myofiber shrinkage and impair protein functionality, leading to reduced water retention of muscles (Wilhelm et al., 2010). When the reduction in water holding capacity is associated with pale color and low ultimate pH, the resulting meat could be referred to as PSE-like meat (Woelfel et al., 2002; Wilhelm et al., 2010). However, cooking losses of commercial broilers slaughtered at 7 and 10 weeks of age were increased compared to 4 week old broilers, despite high ultimate pH values. A recently characterized aberration of breast meat quality from commercial broilers, the white striping defect, was, in contrast to the PSE-like condition, associated with both high pH and elevated cooking losses (Mudalal et al., 2014; Mudalal et al., 2015). It has been suggested that the physiological reason for the white striping defect and/or reduced cooking yields are reductions in myofibrillar and sarcoplasmic protein concentrations of breast meat (Mudalal et al., 2014). This could have resulted in less protein-bound and immobilised water and more free water within myofibres (Pearce et al., 2011) causing a reduced ability of the meat from older and/or heavier broilers to retain water during cooking compared to meat from the slow-growing lines.

#### **Effects of line and slaughter age on color and hemin concentration of breast meat**

Color primarily contributes to the appearance of meat products and plays an important role for the purchase decisions of consumers, particularly with respect to cut-up poultry products. Because melanin has a strong impact on meat color in SGD birds, their color values were not analyzed. When compared at equal age, differences in meat color assessed on the dorsal fillet surface of both light-skinned lines were negligible. Yet, the color of the dorsal fillet surface of 4 week old FGL birds clearly diverged from the SGL chickens and was significantly brighter and yellower, more intense and exhibited a significantly greater hue angle. Other studies found that slow-growing genotypes revealed darker (Berri et al., 2001; Sirri et al., 2011), but, in contrast to the present results, redder, and yellower breast meat (Berri et al., 2001; Sandercock et al., 2009). According to N'dri et al. (2007), the  $b^*$  values of breast meat of broilers did not differ from a slow-growing line when reared under normal ambient temperatures, but they were increased for broilers at high ambient temperatures. Sirri et al. (2011) reported that  $b^*$  values were not differing between a broiler and a medium-growing line at equal age, but  $a^*$  values were significantly elevated for broiler breast meat. Schneider et al. (2012) observed redness of breast meat of younger broilers was increased compared to older broilers, whereas for  $L^*$  values this trend was reversed. Baéza et al. (2012) found no consistent age-related trend for the redness of breast meat of a commercial broiler. The scattering and refraction of light through the surface and deeper layers of meat are closely related to the extent of pH decline and the associated alterations in the spacing of the myofibril lattice (Swatland, 2008). Thus, the relationship of reduced fillet lightness with increased maximum growth rate observed for the broiler chickens could result from simultaneous changes in ultimate pH values. Chicken breast meat redness is influenced by the myoglobin and hemoglobin concentration in the muscle (Boulianne and King, 1998). Berri et al. (2001) detected lower heme-iron levels in selected chicken lines compared to their unselected counterparts, suggesting that selection on growth could result in declined levels of hemic pigment. In contrast, we did not find differences in hemin concentrations in breast meat of



broilers compared to SGL birds. Overall, the differences in color between SGL and FGL chickens slaughtered at an equal age, and in the hemin concentration between all three investigated lines, were small, and do not suggest a close relationship between growth rate and hemin concentration in chicken breast muscle.

### **Potentially confounding factors and implications**

In the present work, the year and its interaction with the experimental treatments (line-age combinations) had effects on most growth, carcass and meat quality traits. Altered rearing and (pre-) slaughter conditions may influence meat quality in a genotype-specific manner and have to be critically considered in view of the significant year effect and its interactions with line-age. These confounding variables could also be relevant with respect to contradictory results among different studies. However, in case of the growth and carcass characteristics, the large effect sizes noted for the treatment factor allowed the reproduction of the results in two consecutive years despite the presence of significant interactions. The same applied to the cooking loss of breast meat. For the other functional properties of the breast meat no significant interactions between year and line-age were noted. In contrast, except for the lightness of breast meat, clear statements in regard to line-age effects on most of the color parameters were prevented due to small effect sizes and the presence of significant interactions.

Several studies pointed towards a higher stress-susceptibility of chicken strains exhibiting high growth rates, as indicated by increased creatine kinase levels (Branciarri et al., 2009; Sandercock et al., 2009). Thereby, particularly under conditions of acute heat stress, creatine kinase activity increased in a broiler line compared to layer chickens, possibly resulting in compromised sarcolemmal integrity (Sandercock et al., 2006). Because our experiment was carried out in the summer months, interactions between ambient temperature and line have to be taken into account and could have contributed to the increased cooking losses of the broiler, particularly the extremely high temperatures in July 2013. Altogether, the external factors such as high ambient temperature during rearing and holding, stress-reduced handling (Berri et al., 2005), and early deboning (Mehaffey et al., 2006) could have been somewhat favorable for the expression of meat quality of slow-growing chickens which has to be considered when interpreting the results.

In conclusion, functional breast meat quality varied in certain aspects when comparing slow-growing chicken lines to a fast-growing broiler, but the differences were age- and/or body weight-dependent. The results suggest that selection on high growth rates, particularly of the pectoral muscle, applied to commercial broilers (i.e. their purebred great-grandparental lines), could be primarily associated with increased pH when the body weight of the birds reaches about 40 to 60% of their growth potential, and, possibly, also with impaired water retention of meat during cooking. This situation resembles the recently described white striping defect of poultry meat. But, within each of the investigated chicken lines, no adverse correlations of growth curve parameters with breast meat quality were detected. This contradicts the statement that an increased growth rate is per se detrimental for meat quality. Interestingly, the correlation of ultimate pH and cooking loss with peak growth rate were either significant for the fast-growing line or both slow-growing lines, pointing towards a non-linear relationship of these variables. Thus, breast meat functionality in fast-growing broilers could still be safeguarded by breeding birds for high growth potential, albeit, this might not necessarily contribute to improved production efficiency and profitability. This study further implicates that factors inherent to the individual bird, such as genotypic growth rate and growth potential, are relevant for

the expression of meat functionality, but the interactions of line with slaughter age and the conditions during rearing, slaughtering and processing have to be carefully considered. This was exemplified by the often highly significant interactions with year effects.

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### **3 Paper II: Association of colorimetric traits with pH and pigment contents of breast fillets is affected by measuring position, storage and chicken line**

P.C. Muth, A. Valle Zárate

Animal Breeding and Husbandry in the Tropics and Subtropics, Hans-Ruthenberg-Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Garbenstr. 17, 70599 Stuttgart, Germany

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### ***Rationale***

Colorimetric measurements provide information on the reflective properties of poultry meat and relate to functional and biochemical traits, suggesting their application during processing of poultry products and within breeding programs.

### ***Objective and scope***

- However, the relationship of instrumental color values with pH and pigment traits could vary with production and measuring conditions. Therefore, this was evaluated for chicken breast fillets in this study.

### ***Main findings***

- Breast meat fillets of 139 male birds from two lines exhibiting divergent growth potential were used. Directly after deboning the breast muscle and after 24, 72 and 144 h postmortem, the color profile was determined on two positions of the surface of breast fillets. Then, the associations of instrumental color values with ultimate pH and heme pigment levels were evaluated. Generally, reduced L\* and, particularly, b\* values were related to elevated ultimate pH values of chicken breast meat, whereas increased a\* values point towards an augmented heme pigment concentration.
- However, this study showed that the magnitude of phenotypic correlations was influenced by line, measuring position and storage as well as their interactions. Notably, the interaction between measuring position and line requires attention. For example, for a fast-growing line, no significant correlation was detected between L\* values and ultimate pH on the ventral surface of fillets, whereas the correlation between these parameters on the same position was significantly negative for a slow-growing line throughout storage.
- Interestingly, also the association between pigment levels and b\* values of breast meat was genotype-specific.
- Thus, colorimetric data can potentially be used for sorting chicken breast fillets according to technological quality, for process control, and within breeding programs, but line and measuring conditions have to be carefully considered.

### 3.1 Introduction

The importance of poultry meat color for consumer preference has been demonstrated by Kennedy et al. (2005), who pointed out the highly relevant association between color sensation and perception of eating quality. This illustrates why there is an enormous desire in poultry meat research to measure and understand color.

In numerous studies, reflectance colorimeters (also termed 'chromameters') substitute costly sensory panels and are used to noninvasively determine poultry meat color. These instruments are equipped with a lighting source and a group of three photodiodes, which correspond to the cone types of the human eye. In meat research, Minolta-branded instruments are the most commonly used colorimeters, followed by Hunter-branded devices (Tapp III et al., 2011). Among studies measurements differ not only in the brand and model of the instrument, but also in its setting (illuminant, observer angle, aperture size), the measuring position, the blooming time of meat, and the number of repetitions (Tapp III et al., 2011). During colorimetric measurement, the light reflected from an object is translated into standard color coordinates (XYZ color space), defined by the Commission Internationale de l'Éclairage (CIE). These are further converted according to the Cartesian CIE  $L^*a^*b^*$  ( $L^*$ =lightness,  $a^*$ =redness,  $b^*$ =yellowness) or polar  $L^*C^*h^\circ$  ( $C^*$ =chromaticity,  $h^\circ$ =hue) color space. It has been suggested that instrumental color values be utilized in order to separate chicken fillets according to quality categories (Woelfel et al., 2002) and for breeding programs (Le Bihan-Duval et al., 2001; Harford et al., 2014), because of the association of color values with other quality parameters.

Considering the physiological background of (instrumental) meat color, it has been found that the reflectance and transmittance of light through meat is largely dependent on postmortem glycolysis (Swatland, 2008). Recently, it was reported that carotenoids also play an important role in the determination of poultry meat color, particularly for its yellow component (Le Bihan-Duval et al., 2011). Finally, redness of meat is related to the concentration of hemoproteins, mainly myoglobin and hemoglobin, and to their redox forms (Boulianne and King, 1998; Bekhit and Faustman, 2005). For the extraction and quantification of heme pigments, various procedures are available. Petracci and Baéza (2011) suggested Hornsey's method (1956) as standard in order to quantify the concentrations of heme pigment in poultry meat. Alternatively, Krzywicki's method (1982) allows the determination of the concentration of myoglobin and of the relative proportions of its redox forms.

Protocols in poultry meat research and processing need to take various measuring parameters into account in order to exploit the predictive relationships between colorimetric data and the traits of interest. The first objective of the present study was, therefore, to investigate whether the associations of color values with physical and chemical characteristics of chicken breast fillets vary with the point in time during storage and fillet topography. Because in chicken meat production different lines (e.g., fast-growing standard broilers as opposed to slow-growing local breeds for the production according to various label specifications) are used, it was additionally investigated whether relationships between color values with pH and pigment traits of chicken breast meat are influenced by line.

## 3.2 Material and methods

### Animals and sample collection

The experiment was conducted from May until July 2012, and replicated in the period from June until August 2013. In total, 139 male birds from two lines exhibiting divergent growth potentials were used. The slow-growing (SG) line ( $n = 80$ ) was a meat-type chicken line kept by the University of Hohenheim (Stuttgart, Germany) from 2001 to 2014. The line was established through the crossing of the Rhode Island breed with the New Hampshire breed, segregating for the recessive scaleless (*sc*) gene and the naked neck (*Na*) gene. From this line, hatching eggs were collected and incubated on the research station and the sex was determined on day-old chicks by cloacal sexing. The birds were all heterozygous at the *Sc* locus, and, thus, normally feathered. Fast-growing (FG) day old male Ross 308 broilers ( $n = 59$ ) (Aviagen Group, Huntsville, AL, USA) were obtained from a commercial hatchery (Brütereier Weser-Ems GmbH & Co. KG, Regenstauf, Germany). The number of SG chickens was increased compared with the FG birds in order to balance out the animal density and to have a sufficient buffer. All birds were individually identified with numbered wing tags and distributed to one of two pens per line, each with an area of 9 m<sup>2</sup> and stocked at a maximum density of ~5 kg/m<sup>2</sup>. Lighting was provided for 24 h for the first two days post-hatch and subsequently for 18 h per day until the end of the experiment. The birds had ad libitum access to feed and water. A starter diet (metabolizable energy: 12.6 MJ/kg; crude protein: 212 g/kg) was fed from 0-3 weeks of age, and a wheat- and soybean meal-based grower diet (metabolizable energy: 12.5 MJ/kg; crude protein: 194 g/kg) from 3-10 weeks of age. At 7 and 10 weeks of age, each 40 birds of the SG line were slaughtered, whereas 30 and 29 FG broilers were slaughtered, respectively. Feed was withdrawn approximately 12 h before slaughter. After electrical stunning (110 mA), the neck was cut and the birds bled. The carcasses were scalded in a 65°C water bath for 20 s and defeathered in a rotary drum picker for 10 s, then eviscerated manually and, after chilling for 2 h at 4°C, the breast muscle was deboned. From the right muscle, 30 g meat samples were harvested at a medial position and stored in plastic tubes at -20°C for pigment analyses. The remaining muscle was stored refrigerated in sealed plastic bags at 4°C and used for the determination of breast meat color and pH.

### pH and color measurement

At 24 h postmortem, the ultimate pH (pHu) of the left breast muscle was recorded with a pH meter (InoLab pH Level 1, WTW GmbH, Weilheim, Germany), equipped with a glass electrode (SenTix SP, WTW GmbH, Weilheim, Germany) and calibrated prior to use at pH 4.0 and 7.0. Duplicate measurements at 1 cm depth on the medial portion of each fillet were averaged.

Directly after deboning the breast muscle (2 h postmortem) and at 24, 72 and 144 h postmortem, the color profile was determined on the cranial (upper) portion of the ventral (adjacent to the skin) and dorsal (adjacent to the bone) surface of breast fillets. A Minolta colorimeter (CR-400, Konica Minolta Sensing Inc., Tokyo, Japan) with an illuminant C, 2° observer angle and 8 mm aperture size was used. Prior to each use, the colorimeter was calibrated using a white ceramic tile (Reference No. 13133117,  $Y=87.1$ ,  $x=0.3164$ ,  $y=0.3237$ ). For each measuring position (ventral and dorsal), eight non overlapping readings were averaged. Values were reported according to the CIE L\*a\*b\* color space (L\*: 0 black, 100 white; a\*: <0 green, >0 red; b\*: <0 blue, >0 yellow).



## Heme pigment determination

The determination of soluble myoglobin (SM) was carried out in duplicate for each sample. The method suggested by Krzywicki (1982) and Faustman and Phillips (2001) was slightly further modified. Slices ( $5 \pm 0.02$  g) of the thawed right breast muscle were minced. To each sample, 20 mL of deionized water was added. The mixture was homogenized twice at 9,000 rpm and 13,500 rpm for each 30 s on ice. After centrifugation at  $2,500 \times g$  for 10 min at  $5^\circ\text{C}$ , the supernatant was filtered through a wetted Whatman<sup>TM</sup> filter type GF/C (GE Healthcare, Little Chalfont, UK) by vacuum filtration. Then 2 mL of the filtrate was centrifuged at  $22,000 \times g$  for 25 min at  $5^\circ\text{C}$ . The absorbance was read by a spectrophotometer using an acryl cuvette of 1 cm path length at 730, 582, 557, 525 and 503 nm. At a wavelength of 730 nm, the homogenate is considered to be pigment free, thus allowing correction for sample turbidity. Ten samples exceeding an absorbance of 0.01 at 730 nm were discarded. The concentration of SM in wet tissue was calculated, modified after Van Laack et al. (1996):

$$\text{SM (mg/g)} = (A_{525} - A_{730}) / 7.6 \times 17,291 \times \text{dilution factor},$$

where  $A_{525}$  = absorbance at a wavelength of 525 nm;  $A_{730}$  = absorbance at a wavelength of 730 nm; 7.6 = millimolar extinction coefficient ( $\epsilon_{\lambda}$ /mM/cm) at 525 nm (Bowen, 1949); 17,291 = molecular mass of myoglobin in Da (Maheswarappa et al., 2009).

The concentration of the redox forms of metmyoglobin (MetMb), oxymyoglobin (OxyMb) and deoxymyoglobin (DeoMb) were calculated according to Tang et al. (2004) based on the absorbances at 582, 557, 525 and 503 nm and expressed in  $\mu\text{g}/\text{mg}$  of myoglobin.

The determination of hemin concentration was carried out in duplicate for each sample. The procedure was conducted according to Hornsey (1956) and Carpenter and Clark (1995) with slight modifications. Slices ( $5 \pm 0.02$  g) of the right breast muscle were minced and mixed with 20 mL acetone and 0.5 mL HCl. Water was added until total water, including the water content of the meat, equaled 4.5 g. The mixture was homogenized twice for 15 s on ice. The tube was capped and held for at least 1 h under subdued light at  $4^\circ\text{C}$ . After centrifugation at  $2,500 \times g$ , 2 mL of the supernatant was removed and centrifuged at  $20,000 \times g$ . The samples were then transferred into quartz glass cuvettes of 1 cm path length, and absorbance was read at 640 and 730 nm. Hemin content on a wet tissue basis was calculated as follows:

$$\text{Hemin (ppm)} = (A_{640} - A_{730}) / 4.8 \times 652 \times \text{dilution factor},$$

where  $A_{640}$  = absorbance at a wavelength of 640 nm;  $A_{730}$  = absorbance at a wavelength of 730 nm; 4.8 = millimolar extinction coefficient ( $\epsilon_{\lambda}$ /mM/cm) at 640 nm (Hornsey, 1956); 652 = molecular mass of hemin in Da (Hornsey, 1956).

## Statistical analysis

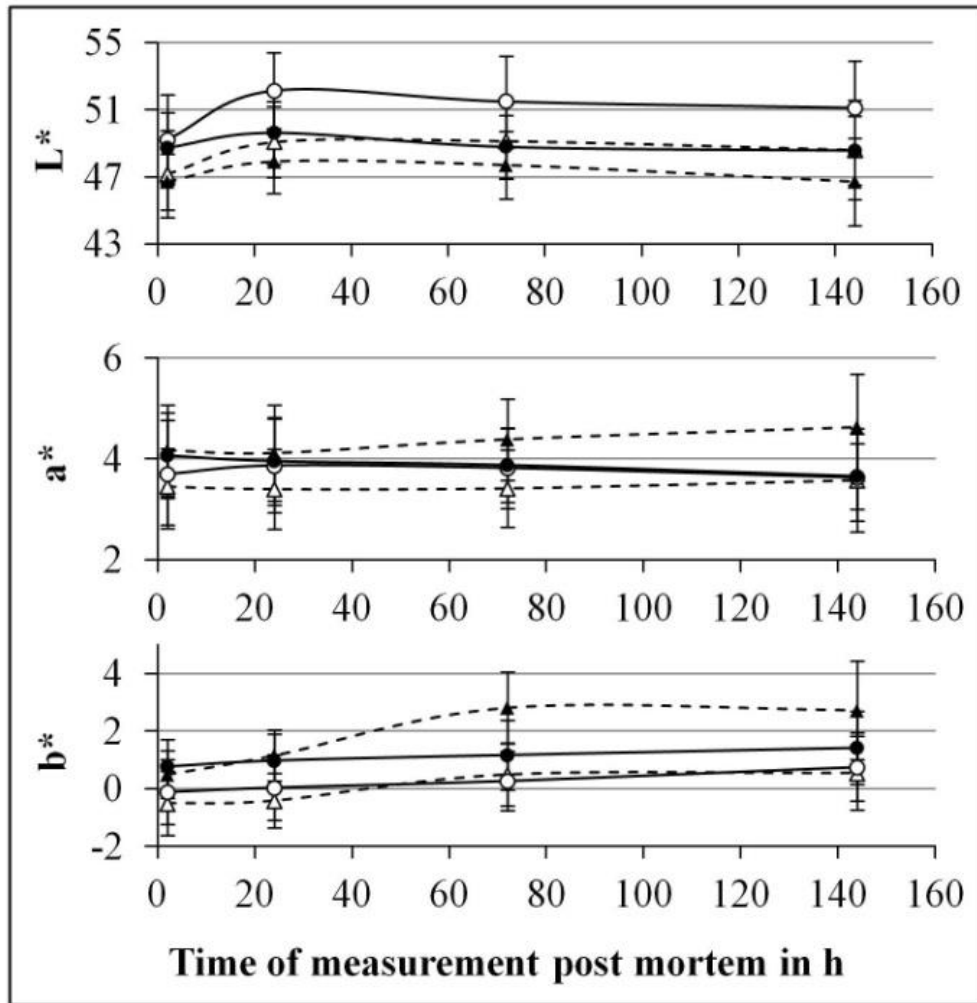
The experimental design comprised a temporal replicate (years 2012 and 2013) and slaughter groups at two different ages (7 and 10 weeks). The breast muscle of an individual bird represented the experimental unit. Descriptive statistics for pH, pigment traits and color values (CIE L\*a\*b\*) are summarized in Table 3.1 and in Figure 3.1, respectively.

**Table 3.1** Mean and standard deviation (SD) for pH values at 24 h postmortem (pHu), hemin concentration, soluble myoglobin concentration (SM), and the contribution of redox forms (metmyoglobin, MetMb; oxymyoglobin, OxyMb; deoxymyoglobin, DeoMb) to total soluble myoglobin concentration in chicken breast fillets<sup>a</sup>

Trait	Fast-growing line			Slow-growing line		
	N	mean	SD	N	mean	SD
pHu	59	5.84	0.13	80	5.71	0.09
Hemin (ppm)	59	9.63	2.83	78	10.25	2.86
SM (mg/g)	52	0.159	0.006	77	0.169	0.005
MetMb (µg/mg)	51	463	63	77	425	62
OxyMb (µg/mg)	51	445	95	77	505	97
DeoMb (µg/mg)	51	116	44	77	87	49

<sup>a</sup>Breast fillets were derived from male chickens of a fast-growing line (n = 59) and a slow-growing line (n = 80) slaughtered at 7 and 10 weeks of age

The calculation of Pearson product-moment correlation coefficients (r) for the statistical relationships between color values, ultimate pH and pigment traits was based on the residuals of linear models correcting for the effects of age and replication (year). Correlation coefficients of residuals were calculated for each combination of line, measuring position and the point in time. R software version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria, 2014) was used for the statistical analysis.



**Figure 3.1:** Meat color (mean and SD of CIE L\*a\*b\* values) of male chickens of a fast-growing line (n = 59; circles and solid line) and a slow-growing (n = 80; triangles and dashed line) line slaughtered at 7 and 10 weeks of age during storage, compared for the ventral (skin-side; open symbols) and dorsal (bone-side; solid symbols) surface of breast fillets.

### 3.3 Results

For both chicken lines considered in this research pHu values were significantly negatively related to L\* and, particularly, b\* values (Table 3.2). For SG birds, the residual relationship between L\* and b\* values with pH values was significant for both surfaces of the fillet and almost all points in time throughout storage, except for L\* values at 2 h postmortem measured on the ventral side. In contrast, the association of L\* values with pHu was only significant on the dorsal side of the fillets for FG broilers. Comparing the dorsal surfaces, this association was similar in magnitude for both lines. No significant relationships were detected between a\* values and pH for SG birds. For FG birds, a\* values assessed on the dorsal side at 2 h postmortem and pHu were negatively correlated ( $P < 0.05$ ).

**Table 3.2:** Pearson product-moment residual correlations between pH values at 24 h postmortem and instrumental color values (CIE L\*a\*b\*) of chicken breast fillets during storage, for the ventral (skin-side) and dorsal (bone-side) surface of fillets<sup>a</sup>

Position	Time of measurement post mortem							
	2 h		24 h		72 h		144 h	
	ventral	dorsal	ventral	dorsal	ventral	dorsal	ventral	dorsal
SG								
L*	NS	-0.295*	-0.238*	-0.300**	-0.323**	-0.451**	-0.283*	-0.437**
a*	NS	NS	NS	NS	NS	NS	NS	NS
b*	-0.297**	-0.336**	-0.500**	-0.451**	-0.570**	-0.598**	-0.571**	-0.373**
FG								
L*	NS	-0.310*	NS	-0.310*	NS	-0.398**	NS	-0.366**
a*	NS	-0.257*	NS	NS	NS	NS	NS	NS
b*	NS	-0.436**	-0.263*	-0.313**	NS	-0.545**	NS	-0.432**

\*, \*\* indicates significance of correlation coefficients at  $P < 0.05$  and  $P < 0.01$ , respectively; NS = not significant.

<sup>a</sup>Breast fillets were derived from male chickens of a fast-growing line ( $n = 59$ ) and a slow-growing line ( $n = 80$ ) slaughtered at 7 and 10 weeks of age.

Several associations between a\* values and the SM concentration of chicken breast fillets were detected (Table 3.3). In summary, for six out of eight and five out of eight combinations of measuring position and point in time significant positive residual correlations were observed for the SG and FG line, respectively. For SG chickens, also significantly negative residual correlations between L\* values and SM concentrations were noted for the dorsal surface from the early phase postmortem until day three of storage. L\* values measured on the ventral surface of breast fillets stored for three and six days also tended to be negatively related to SM concentrations ( $r = -0.200$  and  $-0.202$ , respectively;  $P < 0.1$ ; data not shown). In contrast, SM concentration exhibited no association with lightness of fillets from FG broilers. Interestingly, relationships for b\* values were different comparing lines. From 24 h postmortem onwards, FG birds revealed significantly positive coefficients for the relation of b\* values determined on the ventral surface of fillets and SM concentrations, whereas the relationships between these parameters were not significant or even negative for fillets of SG birds.

**Table 3.3:** Pearson product-moment residual correlations between soluble myoglobin concentration and instrumental color values (CIE L\*a\*b\*) of chicken breast fillets during storage, for the ventral (skin-side) and dorsal (bone-side) surface of fillets<sup>a</sup>

Position	Time of measurement post mortem							
	2 h		24 h		72 h		144 h	
	ventral	dorsal	ventral	dorsal	ventral	dorsal	ventral	dorsal
SG								
L*	NS	-0.302**	NS	-0.356**	NS	-0.248*	NS	NS
a*	NS	0.260*	NS	0.335**	0.313**	0.335**	0.266*	0.276*
b*	NS	NS	-0.226*	NS	NS	-0.231*	NS	NS
FG								
L*	NS	NS	NS	NS	NS	NS	NS	NS
a*	0.312*	NS	0.314*	0.331*	0.287*	NS	0.279*	NS
b*	NS	NS	0.302*	NS	0.324*	NS	0.304*	NS

\*, \*\* indicates significance of correlation coefficients at  $P < 0.05$  and  $P < 0.01$ , respectively; NS = not significant.

<sup>a</sup>Breast fillets were derived from male chickens of a fast-growing line ( $n = 52$ ) and a slow-growing line ( $n = 77$ ) slaughtered at 7 and 10 weeks of age.

The association of hemin levels with a\* values was significantly positive for most measuring position and time point combinations for fillets of both chicken lines, except for the dorsal surface of fillets from SG chickens at 2 h postmortem and on the ventral surface of fillets from FG broilers on the days three and six of storage (Table 3.4). Once again, hemin concentrations were positively related to b\* values of fillets of FG broilers on both surfaces of the fillets, particularly at days three and six of storage. For SG birds, the relationship between these parameters was either not significant or even significantly negative on the dorsal surface of the fillet at day six of storage ( $P < 0.01$ ). Hemin contents were not related to the lightness of breast fillets of either line.

**Table 3.4:** Pearson product-moment residual correlations between acidified hemin concentration and instrumental color values (CIE L\*a\*b\*) of chicken breast fillets during storage, for the ventral (skin-side) and dorsal (bone-side) surface of fillets<sup>a</sup>

Position	Time of measurement post mortem							
	2 h		24 h		72 h		144 h	
	ventral	dorsal	ventral	dorsal	ventral	dorsal	ventral	dorsal
SG								
L*	NS	NS	NS	NS	NS	NS	NS	NS
a*	0.291*	NS	0.354**	0.318**	0.305**	0.250*	0.385**	0.275*
b*	NS	NS	NS	NS	NS	NS	NS	-0.436**
FG								
L*	NS	NS	NS	NS	NS	NS	NS	NS
a*	0.352**	0.395**	0.353**	0.304*	NS	0.324*	NS	0.289*
b*	NS	NS	0.373**	NS	0.361**	0.302*	0.307*	0.367**

\*, \*\* indicates significance of correlation coefficients at  $P < 0.05$  and  $P < 0.01$ , respectively; NS = not significant.

<sup>a</sup>Breast fillets were derived from male chickens of a fast-growing line ( $n = 59$ ) and a slow-growing line ( $n = 78$ ) slaughtered at 7 and 10 weeks of age.

For both chicken lines, and at most points in time during storage, the fraction of MetMb was negatively related to a\* values (Table 3.5), particularly on the ventral side of fillets. The b\* values measured at 2 h postmortem on the ventral surface of the breast fillets from FG birds were significantly positively associated with the proportion of MetMb ( $P < 0.05$ ). In contrast, the residual relationship of b\* assessed at 72 h ( $P < 0.01$ ) and at 144 h ( $P < 0.1$ ) postmortem with MetMb was negative for the dorsal surface. Since the estimation of the proportion of OxyMb and DeoMb is based on the absorbance at the same wavelengths as of MetMb, similar magnitudes but opposite signs have been obtained for the correlations of color values with OxyMb, whereas for the association with DeoMb, similar correlations compared with those for MetMb were yielded (data not shown).

**Table 3.5:** Pearson product-moment residual correlations between proportion of metmyoglobin and instrumental color values (CIE L\*a\*b\*) of chicken breast fillets during storage, for the ventral (skin-side) and dorsal (bone-side) surface of fillets<sup>a</sup>

Position	Time of measurement post mortem							
	2 h		24 h		72 h		144 h	
	ventral	dorsal	ventral	dorsal	ventral	dorsal	ventral	dorsal
SG								
L*	NS	NS	NS	NS	NS	NS	NS	NS
a*	-0.321**	NS	-0.334**	-0.282*	-0.290*	NS	-0.258*	NS
b*	NS	NS	NS	NS	NS	NS	NS	NS
FG								
L*	NS	NS	NS	NS	NS	NS	NS	NS
a*	NS	-0.317*	-0.298*	NS	-0.342*	-0.331*	-0.309*	NS
b*	0.308*	NS	NS	NS	NS	-0.387**	NS	NS

\*, \*\* indicates significance of correlation coefficients at  $P < 0.05$  and  $P < 0.01$ , respectively; NS = not significant.

<sup>a</sup>Breast fillets were derived from male chickens of a fast-growing line ( $n = 51$ ) and a slow-growing line ( $n = 77$ ) slaughtered at 7 and 10 weeks of age.

The residual correlation between the hemin content determined according to Hornsey (1956) and the myoglobin concentration determined according to Krzywicki (1982) tended to be significant for SG chickens ( $r = 0.213$ ;  $P < 0.1$ ; data not shown). For FG broilers a moderate correlation between both extraction techniques was found ( $r = 0.428$ ;  $P < 0.01$ ; data not shown). Ultimate pH values were not related to hemin levels in breast fillets of both lines, but for FG broilers a significant positive association between SM concentration and pH<sub>u</sub> was recorded ( $r = 0.342$ ;  $P < 0.05$ ; data not shown). The relationships between pH and myoglobin redox forms were also highly significant for FG birds. Thereby, pH<sub>u</sub> was positively correlated with MetMb ( $r = 0.652$ ;  $P < 0.01$ ; data not shown) and DeoMb ( $r = 0.534$ ;  $P < 0.01$ ; data not shown), but negatively related to the fraction of OxyMb ( $r = -0.628$ ;  $P < 0.01$ ; data not shown). For SG chickens ultimate pH correlated significantly positively to the fraction of DeoMb ( $r = 0.270$ ;  $P < 0.05$ ; data not shown).

### 3.4 Discussion

The association of rate and extent of postmortem acidification with instrumental color values has been extensively investigated. In accordance with the residual correlations presented herein, ultimate pH was found to be negatively correlated with lightness (Wilkins et al., 2000; Qiao et al., 2001; Berri et al., 2005; Janisch et al., 2011) and yellowness (Wilkins et al., 2000; Qiao et al., 2001; Janisch et al., 2011), measured on the ventral (Wilkins et al., 2000; Berri et al., 2005) or dorsal (medial) surface (Qiao et al., 2001; Janisch et al., 2011) of breast fillets. Swatland (2008) showed that lactate accumulation and postmortem pH fall straitened the spacing of the intracellular myofibrillar lattice and altered the reflective and transmissive properties of meat. Thereby, the light path through meat could be decreased, limiting the absorbance of light by pigments, while reflectance and light scattering could be increased (Swatland, 2008), which well explains the elevated instrumental lightness values at reduced pH. The present experiment supports the assumption that colorimetric color values can provide information on the pH of chicken breast meat, whereas high L\* and b\* values indicate a low pH of meat. But the results also suggest that some considerations are necessary

before implementing colorimetric data as an indicator for pH. Firstly, the measuring position in relation to the time point of measurement is important. It appears that in case of early deboning, color measurements should be performed on the dorsal surface (bone-side) of the fillet. Meat color before completion of *rigor mortis* could rather be related to the onset of acidification (Berri et al., 2005), explaining why L\* values assessed at 2 h postmortem on the ventral side were not correlated to the ultimate pH of fillets. Despite the fact that meat color changes over time (Berri et al., 2001; Janisch et al., 2011), the relationship between the L\* and b\* values measured on different points in time during storage and pH assessed at 24 h postmortem remained quite stable. This is indicating the feasibility to predict ultimate pH from color values recorded within six days of storage. Secondly, the association of L\* and b\* with pH was also dependent on the line in relation to the measuring position. Throughout storage, the measurement of lightness on the ventral surface of the fillet was not associated with pH for the FG broiler, implying that, for this line, instrumental color values assessed on the ventral surface hardly provide information on the pH of the meat. In contrast, recording L\* values on the dorsal surface of fillets might be suitable for the prediction of ultimate pH values in both lines. It has been shown before that colorimetric values varied according to the measuring position on the ventral surface of fillets (Wilkins et al., 2000), but also when comparing the ventral and dorsal position of the fillet (Muth and Valle Zárate, 2013). This could explain inconsistent results when comparing correlations among measuring positions for the FG line. Finally, the genotype-specific variation in the reflective properties among fillet surfaces could cause substantial interference in the evaluation of the association of colorimetric data with pH.

In contrast to the association between ultimate pH and color values, less literature exists on the relationship between colorimetric traits and the heme pigment content of chicken breast meat. Heme pigment concentrations in chicken breast fillets were low according to both extraction methods, and yielded 0.17 mg/g SM or 9.98 ppm hemin (0.88 ppm heme-bound iron). Assuming a molecular weight of 17,291 Da for myoglobin and 64,500 Da for hemoglobin, levels of 0.26 mg/g myoglobin or 0.99 mg/g hemoglobin can be inferred from the hemin concentration. As the breast muscle of chicken is (almost) entirely composed of type IIB glycolytic myofibers (Branciarri et al., 2009), low concentrations of heme pigments were expected. Thus, heme pigments appear to be present primarily in the form of hemoglobin, possibly due to incomplete bleeding of breast muscles after slaughter (Alvarado et al., 2007), or because of increased blood circulation due to wing flapping activity on the shackle line (Berri et al., 2005). In this study the birds were stunned individually under stress-reduced conditions and pigment concentrations in the breast muscle were low and comparable among both lines.

Both extraction methods confirmed that the concentration of heme pigments in chicken breast fillets contributes positively to a\* values. Both procedures provided similar results and could be suggested for pigment extraction from chicken breast meat. However, it appears that SM determination could be confounded with the pH of the meat. Moreover, the strength of the correlations was rather low, with the coefficient of determination being mostly below 0.15. Indeed, Van Laack et al. (1996) found that in pig loin samples, the strength of the association between hemin concentration and a\* values decreased with hemin content; thus, at low pigment levels, both extraction methods may lack precision. In accordance with our results, Boulianne and King (1998) detected a strong positive association between a\* values measured at 1 h postmortem and the heme pigment content ( $r = 0.75$ ) of chicken breast, but also a negative relationship of L\* values with the pigment concentration of the fillets ( $r = -0.69$ ). However, they pre-selected samples according to their appearance (300 dark



and 300 normal samples) resulting in an overrepresentation of dark fillets, which might be the primary reason for the high coefficients.

The associations between heme pigment levels with  $a^*$  values were largely unaffected by the measuring position, storage and chicken line. In contrast, the association between pigment levels and  $b^*$  values of breast meat was genotype-specific. For FG broilers, significant positive correlations were detected, especially if pigment levels were extracted according to the Hornsey (1956) method and for  $b^*$  values recorded in the late phase of storage, while for SG birds, these associations were mostly not significant or even negative. The changes in the relationships particularly of the  $b^*$  value with pigment traits for different points in time could be related to discoloration due to metmyoglobin (or methemoglobin) accumulation in the layers close to the surface of broiler breast fillets during storage (Ryu et al., 2005). Different rates of oxygen consumption and/or metmyoglobin (or methemoglobin) reducing capacity could also underlie the genotype-specific correlations of  $b^*$  values with heme pigment concentrations observed herein. Applying the equations of Tang et al. (2004) it was estimated that about 440  $\mu\text{g}/\text{mg}$  of the heme pigment in chicken breast fillets of both lines was oxidized, 481  $\mu\text{g}/\text{mg}$  was present in the oxygenated form, and about 99  $\mu\text{g}/\text{mg}$  was deoxygenated. The ultimate pH of chicken breast meat was positively related to the proportion of MetMb in the present study. In contrast, Gutzke and Trout (2002) showed that autoxidation increased with reduced pH, indicating that the estimation of redox forms herein has to be interpreted with caution. Interestingly, an effect of storage on the association of the relative amounts of heme pigment redox forms with  $b^*$  values assessed on fillets from FG birds was detected, which could have been related to color development due to interconversion of redox forms during storage. To explain the differences in correlations between lines, the confounding effects of other factors besides discoloration have to be considered. For instance, the retention of sarcoplasmic pigments during storage (Muth and Valle Zárate, 2013) and the concentration of lutein and zeaxanthin (Le Bihan-Duval et al., 2011) could play a role. The concentration of carotenoids in chicken breast muscle was shown to be associated with the expression of  $\beta$ -carotene 15, 15'-monooxygenase (Le Bihan-Duval et al., 2011), which could result in genetic differences in the color of chicken breast fillets.

In conclusion, instrumental color values of chicken breast meat were associated with ultimate pH as well as pigment levels as determined by two extraction techniques. Thereby, reduced  $L^*$  and, particularly,  $b^*$  values indicate elevated pH values of chicken breast meat, whereas increased  $a^*$  values point towards an augmented heme pigment concentration. Thus, colorimetric data can potentially be used for sorting fillets according to technological quality, or for process control (e. g. of bleeding efficiency). According to the present results, the phenotypic residual correlations of instrumental color values with pH and pigment traits were rather weak and influenced by line, measuring position and storage period, as well as by their interactions. This demands careful consideration when planning research and regarding the practical application of colorimetry in poultry meat processing. In accordance with Petracci and Baéza (2011), we recommend conducting colorimetric measurements on the dorsal (bone-side) of chicken breast fillets to obtain meaningful results.

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#### **4 Paper III: Discriminating the quality of local pork from crossbred pork from extensive production of ethnic minorities in the Southeast Asian Massif**

P.C. Muth<sup>1</sup>, A. Markemann<sup>1</sup>, L.T.T. Huyen<sup>2</sup>, A. Valle Zárate<sup>1</sup>

<sup>1</sup>Animal Husbandry and Breeding in the Tropics and Subtropics, Hans-Ruthenberg-Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Garbenstr. 17, 70593 Stuttgart, Germany

<sup>2</sup>Department of Economics and Livestock Farming Systems, National Institute of Animal Science, Thuy Phuong, Bac Tu Liem, Hanoi, Vietnam

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### **Rationale**

Developing short food supply chains for products from local pig (*Sus scrofa domestica*) breeds may offer chances for smallholders in rural areas to participate in niche markets and obtain additional income

### **Objective and scope**

- Because the authenticity and distinctness of products are crucial for establishing marketing, this study compared selected product characteristics of pork from the Vietnamese Ban breed with pork from crossbreds, each derived at their typical market weight and from their respective extensive and semi-extensive production environment; thus resembling the combination, the products are available for customers.

### **Main Findings**

- Traditional Ban pork could be effectively discriminated from crossbred pork through cut dimensions, exemplified by the significantly reduced loin eye area ( $P < 0.001$ ), and by the significantly reduced backfat thickness ( $P < 0.001$ ).
- Also, marbling fat was significantly decreased in local pork ( $P < 0.001$ ), whereas differences in further meat quality parameters were rather weakly expressed.
- The significantly higher share of polyunsaturated fatty acids in loins of traditionally produced Ban pigs ( $P = 0.003$ ) could possibly result in a preferred nutritional value, but lower oxidative stability of the products.
- This study provides novel information to help improve vertical coordination of value chains for specialty pork in Vietnam and comparable situations in the Southeast Asian Massif.

## 4.1 Introduction

The Southeast Asian Massif (SEAM) covers an area of 2,500,000 km<sup>2</sup> (north-eastern India, Southeast Asia and four Chinese provinces) and there are estimated to live approximately 100 million minority people (Scott, 2009). These societies are culturally and linguistically highly diverse, but they share a state of marginality and forms of subordination to the powerful lowland states (Michaud, 2010). Promoting market-oriented agriculture by cultivating a cash crop and/or intensifying staple crop production yielded some successes with respect to the economic development of marginalized smallholders. But, focusing on one main crop species may increase production risks and vulnerability due to climatic conditions, pests and the fall of market prices (Sturgeon et al., 2013). Therefore, risk mitigation by diversification into a multiplicity of activities and income sources including handicraft, trading, services and animal production can represent essential livelihood strategies for the rural population of this area (World Bank, 2012).

In the SEAM, pig keeping is a widespread activity of rural households. For instance, in Vietnam pigs are kept by more than 80% of rural households in the mountainous regions (Roland-Holst et al., 2010). Thus, pork production could represent a chance for smallholders to participate in growing markets for animal-based products and diversify their incomes. However, the prospects for smallholders to commercialize pig production, specifically those located in the less accessible uplands remain limited because they are disadvantaged by an array of market-related barriers, preventing them from entering the commodity market. Therefore, smallholder pig husbandry in the SEAM is often still subsistence-oriented and based on extensively managed local breeds thriving on low-cost feeding (e.g., Riedel et al., 2012). In the northern mountainous regions of Vietnam, the Ban breed (different names exist) is one of the most widely spread local breeds. Ban pigs are mainly black-coated with small erect ears, and have a low productive and reproductive potential, but are robust and adapted to harsh environments. Similar phenotypic characteristics have been reported for local pig populations in the uplands of south-western China (Riedel et al., 2012), Laos (Phengsavanh et al., 2010), and northern Thailand (Nakai, 2008). In contrast, where favorable market conditions prevail, the application of high-yielding exotic breeds in crossbreeding schemes could help to better match increasing consumer demand for lean pork. In fact, crossbreeding schemes have been adopted by smallholders in the lowlands and those close to provincial centers (e.g., Luc et al., 2014).

Besides the demand for 'regular' lean meat, specific groups of urban consumers more recently expressed preferences for pork from local pigs reared without industrial feed (Lapar et al., 2010). On special occasions, such as the Lunar New Year celebrations, the demand for local pork is at its highest, implying a cultural background for these preferences. Particularly pigs slaughtered at a body weight of less than 20 kg fetch high prices on the markets of southwest China (Neo and Chen, 2009) and northern Vietnam (Le et al., 2016). The niche marketing of value added animal-based products has been identified as a development option for small-scale farmers, but for the smallholders of the SEAM creating marketing options for specialty pork represents a challenge. Information on the unique traits of traditional pork products and its markets is lacking, preventing effective value chain coordination and marketing. First reports demonstrate that target markets could be represented by specialty restaurants or 'green' food shops in the densely populated lowlands (Le et al., 2016). However, consumers may not be willing to pay a premium price for pork products from local breeds unless they can rely on the authenticity of the product and its distinctness from commodity production (FAO, 2013).

The objective of this study was, therefore, to compare selected product characteristics and physico-chemical meat quality traits of traditionally produced pork derived from purebred Vietnamese Ban pigs with pork from crossbred pigs. The study aimed at providing information to improve vertical coordination for emerging value chains for specialty pork in northern Vietnam and comparable situations in the SEAM. Therefore, it was decided that the context of this study should reflect the product categories as available for customers, that is, both genetic groups were raised by smallholder farmers on a management level adapted to the respective genetic resource (extensive for Ban and semi-extensive for crossbreds). Thus, production environments and marketing weights differed largely between genetic groups, however the age at slaughter, slaughter locations and procedures were standardized.

## **4.2 Material and methods**

Transport and slaughtering of live animals (pigs, *Sus scrofa domesticus*) complied with all legal requirements imposed by the Socialist Republic of Vietnam. The trials were conducted under the supervision of the Veterinary Department of Son La province, Vietnam.

### **Study site**

The study was carried out from December 2013 until February 2014 in the city of Son La and its surrounding districts. Son La is the capital of the same-named mountainous province in north-western Vietnam, located on the eastern flank of the SEAM. More than 70% of the population of Son La province is represented by ethnic minorities, predominantly speaking Tai-Kadai or Miao-Yao languages (World Bank, 2009).

### **Animal selection**

In total, 33 purebred Ban pigs and 12 crossbred pigs from Piétrain x Duroc sires (PiDu) were purchased from smallholder farms. The number of Ban pigs slaughtered was increased to generate benchmark data for local pork products in view of the high variability in the husbandry conditions. The semi-extensively produced commercial crossbreds served as reference.

Farms were selected purposively based on the availability of suitable fatteners. All animals were castrated male pigs at an age of 6 to 7 months, each originating from a different litter, dam and farm. For most of the PiDu-sired crossbreds, the dam line was represented by a local breed (e.g., Mong Cai), with a varying degree of genetic admixture with exotic breeds, which could, however, not be determined.

Information obtained from semi-structured questionnaires showed that compared to producers of crossbred pigs, the distance to the in- and output markets of Son La was longer for producers of traditional Ban pork (Table 4.1). Ban keepers also applied less diversified diets and used more fibrous feed components. All farmers cooked the diet before feeding. The way pig keepers prepare and cook the diets was precisely described by Tra (2003). In brief, the farmers usually chopped bulky feedstuffs into small pieces which were then pre-cooked in boiling water before the remaining ingredients were added. The laborious procedure may take up to 2 h. For diet preparation, farmers used on average 4.4 kg fresh matter pig<sup>-1</sup> day<sup>-1</sup> for Ban fatteners and 5.1 kg fresh matter pig<sup>-1</sup> day<sup>-1</sup> for PiDu-sired crossbreds. Husbandry conditions were more diverse for purebred Ban pigs, which were frequently semi-scavenging (i.e. confined only at night) or kept in spacious enclosures. The confounding of genetic with environmental factors with respect to housing and feeding (Table 4.1) were accepted

precisely because of their possible contribution to the final product configuration under real production conditions enabling a comparison of the raw products representative for the market. At slaughter, purebred Ban pigs were, on average, 6.4 months old (range: 6.1 to 6.9 months) and weighed 14.8 kg (range: 6.5 to 26.5 kg), whereas PiDu-sired fatteners were, on average, 6.3 months old (range: 6.0 to 6.8 months) and weighed 58.4 kg (range: 41.0 to 81.5 kg). At this age, products derived from Ban pigs qualify for premium pricing, whereas exotic hogs and their crossbreds are commonly slaughtered at an even higher age and body weight (80 to 100 kg, informal observation).

**Table 4.1** Characterization of production systems in relation to the genetic background (purebred Ban pigs vs. crossbreds from Piétrain x Duroc (PiDu) sires) of products

Trait	Purebred Ban pigs	PiDu-sired crossbreds
	N=33	N=12
Farm distance to regional market (km)	28.7 ± 14.3 (R: 4-45)	8.3 ± 6.5 (R: 0-25)
Number of fatteners kept (N)	4.7 ± 3.0 (R: 1-12)	8.0 ± 4.5 (R: 4-17)
Number of diet ingredients used (N)	2.9 ± 0.8 (R: 2- 4)	4.1 ± 1.0 (R: 3- 6)
Housing type (%)		
Penned	51.5	100.0
Enclosed	15.2	0.0
Semi-scavenging	33.2	0.0
Farmers using a feed item (%)		
Banana pseudostem	90.9	41.7
Rice bran	81.8	58.6
Vegetables (leaves)	39.4	58.3
Maize	30.3	100.0
Brewer's grain	3.0	50.0
Food wastes	6.1	33.3
Fresh matter diet composition (g kg <sup>-1</sup> )		
Banana pseudostem	669	103
Rice bran	117	60
Vegetables (leaves)	89	206
Maize	43	170
Brewer's grain	25	265
Food wastes	24	151

Mean ± standard deviation and range (R) for farm distance to the regional market, number of fatteners kept, number of diet ingredients, percentage for housing type, farmers using a feed item and mean for diet composition (raw materials as added before cooking).



## **Slaughter and sampling**

Three abattoirs representing typical small-scale butchers (capacity of one to two pigs per day, slaughterers are also engaged in retail) applying common slaughter practice, i.e. bleeding without prior stunning, were selected. A maximum of four pigs per day was slaughtered on 13 slaughtering days in total (mixed genetic groups on 10 days). Pigs were collected the day before slaughter and fasted for at least 12 h prior to slaughter. After bleeding, pigs were scalded by pouring hot water over the body and dehaired manually. Subsequent to evisceration, head, feet and leaf fat was removed and then the carcass weight was recorded. The carcass was split along the backbone and backfat thickness was measured at the level of the last rib and above the *m. gluteus medius*. The loin eye area was recorded on the cross-section of the loin between the 13th and 14th thoracic vertebrae. Afterwards, the carcass half was dissected and the lean yield expressed relative to the weight of the dressed carcass half. Samples of the loin (*m. longissimus thoracis et lumborum*, LTL) and ham (*m. semimembranosus*, SM) were excised and dissected, and partly stored refrigerated at 4 °C for meat quality analysis, or frozen at -18 °C for biochemical analysis.

## **Meat quality measurement**

The pH value of fresh meat samples was measured using a hand-held pH meter (pH-STAR, Matthaeus GmbH & Co. KG, Nobitz, Germany), equipped with a glass electrode at 45 min and at 24 h (pH24) postmortem. Prior to each use, the pH meter was calibrated using buffer solutions at pH 4.6 and 7.0. The electrode was inserted to a depth of 2 cm into LTL and SM. Three measurements were averaged for analysis.

The Japanese Color Score (JCS) was determined at 24 h postmortem on the cross-section of the LTL and SM, using a scale ranging from 1 (extremely pale) to 6 (extremely dark red). Meat color (CIE L\*, lightness; a\*, redness; b\*, yellowness) was determined by a CR-400 colorimeter (Konica Minolta Inc., Tokyo, Japan), with a 2° observer angle and illuminant C setting. The colorimeter was calibrated using a white ceramic tile ( $Y = 87.1$ ,  $x = 0.3164$ ,  $y = 0.3237$ ). The average of eight measurements for each sample was used for the analysis.

For the assessment of drip loss after storage, samples of LTL and SM, were weighed and placed in a drainer. The box was tightly sealed with plastic wrap and stored refrigerated at 4 °C until 72 h postmortem. Then, the weight loss of each sample was recorded and expressed relative to the initial sample weight. Cooking loss of LTL and SM, was determined at 24 h postmortem by placing samples in polythene bags and cooking them in boiling water to an internal temperature of 85 °C, controlled by an insertion thermometer. Cooking loss was defined as the weight difference after cooking relative to the sample weight before cooking. Additionally, the water holding capacity of the meat was assessed by the filter paper press method. At 24 h postmortem, small meat samples (~0.3 g) were placed onto filter paper and pressed between two plexiglas blocks for 5 min. Values are given as the ratio of the area of pressed meat over the area of migrated moisture, with higher values indicating an increased water holding capacity. The procedure was repeated three times for LTL and SM.

## **Biochemical analysis**

The determination of water soluble myoglobin was carried out according to Faustman and Phillips (2001) with modifications. Samples ( $5 \pm 0.02$  g) were minced, mixed with 20 mL of distilled water,

homogenized and centrifuged. The resulting supernatant was filtered by vacuum filtration through a Whatman™ microfiber filter grade GF/C (GE Healthcare, Little Chalfont, UK), then centrifuged at 22,000 x g for 25 min. The absorbance was read at 525 and 730 nm, and the concentration of myoglobin in mg per g wet tissue was calculated following van Laack et al. (1996), but without accounting for filtration losses.

The intramuscular collagen level was measured in duplicate on LTL samples according to the AOAC official method 990.26 (AOAC, 1999) for the determination of hydroxyproline with slight modifications. In brief, minced samples ( $4 \pm 0.02$  g) were hydrolyzed and dried at 105 °C for 16 h. The hydrolysate was then filtered and transferred to 100 mL flasks and diluted to volume with distilled water. Subsequently, 200 µL of filtrate was diluted with 1.8 mL water. Oxidant solution and color reagent were added as prescribed. Absorbance was read at 558 nm and the hydroxyproline content of the sample was determined using a calibration curve from hydroxyproline. Collagen content was calculated by multiplying the result with a factor of 8, and expressed relative to the wet sample weight.

The determination of intramuscular fat in LTL and SM samples ( $3 \pm 0.1$  g), was carried out by the Department of Feed and Product Analyses, National Institute of Animal Sciences in Hanoi, Vietnam, using a 1045/1046 Soxtec™ extraction system (FOSS Analytical AB, Hoeganaes, Sweden). The intramuscular fat concentration was determined gravimetrically, and expressed relative to the wet sample weight.

The fatty acid composition of LTL samples was determined by the Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology in Hanoi, Vietnam. Total intramuscular lipids were extracted according to the method of Bligh and Dyer (1959). Fatty acids were then methylated and injected onto a gas chromatograph (model 6890, Hewlett Packard, New York, NY, USA), equipped with a flame-ionized detector and a DB-23 capillary column (Agilent Technologies, Santa Clara, CA, USA). The results are expressed as a percentage of the total fatty acid methyl esters in the sample and summed up to proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The ratio of PUFA to SFA (P/S ratio) and the peroxidizability index, according to Arakawa and Sagai (1986), were additionally calculated.

### **Statistical analysis**

Linear mixed models were fitted using the PROC MIXED function of SAS 9.3 (SAS Institute Inc., Cary, NC, USA). The effects of the genetic group (purebred Ban pigs vs. PiDu-sired crossbreds) and muscle (LTL and SM representing loin and ham, respectively), and their interaction were considered as fixed. Variation due to the slaughter day (13 levels), nested within the slaughter location (three levels), was fitted as a random effect. For drip loss and cooking loss, the initial weight of the sample subjected to measurement was added as a linear covariate. For carcass traits the fixed effect for muscle, and the interaction term were removed from the model, because the effect of muscle did not apply to these traits. Also the models for intramuscular collagen concentration and fatty acid composition simply referred to the fixed effect of genetic group, because the measurements were only conducted on LTL samples. The assumptions of normality of residues and the homogeneity of variances were checked. Five muscle samples (three from purebred Ban pigs and two from PiDu-sired crossbreds) exhibiting dark, firm, dry (DFD) conditions, i.e. pH<sub>24</sub> values > 6.0 had to be excluded from the analysis. Least squares means (LSMEANS statement) were compared pairwise using the PDIFF statement and the

Tukey-Kramer adjustment (ADJUST = tukey) for unbalanced data. The significant level was set at  $P < 0.05$ .

### 4.3 Results

As expected, the lean meat percentage and meatiness, as expressed in the loin eye area, were significantly lower for traditionally produced Ban pigs compared to PiDu-sired crossbreds slaughtered at the same age (Table 4.2). The thickness of the subcutaneous fat layer of Ban hogs was significantly lower compared to the backfat thickness of PiDu-sired fatteners.

**Table 4.2** Carcass characteristics of traditionally raised purebred Ban and crossbred pigs from by Piétrain x Duroc sires

Trait	Purebred Ban		PiDu-sired crossbreds		RSD	Significance <sup>†</sup>
	N	LSM	N	LSM		
Carcass weight (kg)	33	8.2	10	39.6	3.2	***
Lean meat (%)	32	40.9	12	49.5	4.5	***
Backfat at last rib (mm)	33	6.7	12	17.1	3.2	***
Backfat above <i>m. gluteus medius</i> (mm)	33	5.8	11	11.5	3.4	***
Loin eye area (cm <sup>2</sup> )	33	7.6	9	24.2	2.3	***

N - Number of observations, LSM - least squares means, RSD - standard deviation of residuals.

<sup>†</sup> Significance level: \*\*\*,  $P < 0.001$ .

The rate of pH fall was slightly, but significantly decelerated for Ban pigs compared to PiDu-sired crossbreds and in the SM muscle compared to the LTL muscle (Table 4.3). The extent of pH fall, as indicated by pH<sub>24</sub> values, did not differ among genetic groups or among cuts, negating differences in meat acidification. Several samples of both genetic groups exceeded a threshold of  $> 6.0$  in pH<sub>24</sub>, and could therefore be prone to develop the DFD condition.

**Table 4.3** Postmortem glycolysis for muscles (*m. longissimus thoracis et lumborum*, LTL and *m. semimembranosus*, SM) from traditionally raised purebred Ban and pigs from by Piétrain x Duroc (PiDu) sires

Trait	Muscle	Purebred Ban		PiDu-sired crossbreds		RSD	Significance <sup>†</sup>		
		N	LSM	N	LSM		G	M	G x M
pH 45 min	LTL	33	6.32	12	6.20	0.23	*	**	ns
	SM	33	6.55	12	6.36				
pH 24 h	LTL	30	5.57	10	5.60	0.10	ns	ns	ns
	SM	32	5.58	11	5.57				

N - Number of observations, LSM - least squares means, RSD - standard deviation of residuals.

<sup>†</sup> Significance level for the effects of genetic group (G), muscle (M), and their interaction (G x M): \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; ns,  $P > 0.05$ .

Differences in subjective color assessment among pork products derived from different genotypes and production systems were not significant (Table 4.4), but there was a tendency towards lighter, less red Ban loins compared with loins from PiDu crossbreds ( $P < 0.1$ ). The color of the ham of Ban carcasses scored significantly higher (i.e. darker and redder) compared to the loin of Ban pigs. The subjective assessment was backed by instrumental measurements. The SM of purebred Ban pigs revealed lower CIE L\* values, but higher CIE a\* and b\* values than the LTL muscle. Correspondingly, the myoglobin level of hams was significantly higher compared with the loins of purebred Ban pigs. Within carcasses from PiDu-sired hogs, hardly any differences in subjective color scores, instrumental color values, and pigmentation were detected among muscles. Objective meat color did not differ between the loins from purebred Ban pigs and commercial crossbreds, but ham redness and yellowness were significantly higher for purebred Ban fatteners. Levels of water-soluble myoglobin in SM muscle from Ban purebreds tended to be higher compared to those of PiDu-sired crossbred pigs (+21%;  $P < 0.1$ ), which could explain the increased a\* and b\* values of traditionally produced hams.

**Table 4.4** Meat color and myoglobin for muscles (*m. longissimus thoracis et lumborum*, LTL and *m. semimembranosus*, SM) from traditionally raised purebred Ban and pigs from Piétrain x Duroc (PiDu) sires

Trait*	Muscle	Purebred Ban		PiDu-sired crossbreds		RSD	Significance <sup>†</sup>		
		N	LSM	N	LSM		G	M	G x M
JCS	LTL	33	3.10 <sup>y</sup>	12	3.66	0.60	ns	**	*
	SM	33	3.89 <sup>x</sup>	12	3.81				
CIE L*	LTL	33	47.34 <sup>x</sup>	11	46.38	2.36	ns	**	*
	SM	33	43.66 <sup>y</sup>	11	45.49				
CIE a*	LTL	33	8.76 <sup>y</sup>	12	9.04	1.55	ns	***	*
	SM	33	12.05 <sup>ax</sup>	12	10.38 <sup>b</sup>				
CIE b*	LTL	33	2.59	12	1.73	1.02	***	***	ns
	SM	33	3.63	12	2.51				
Mb (mg/g)	LTL	33	0.79 <sup>y</sup>	12	0.92	0.28	ns	*	**
	SM	31	1.12 <sup>x</sup>	12	0.89				

N - Number of observations, LSM - least squares means, RSD - standard deviation of residuals.

\* JCS - Japanese Color Score ranging from extremely light (1) to extremely dark red (6), CIE L\* - lightness, CIE a\* - redness, CIE b\* - yellowness, Mb - water-soluble myoglobin.

<sup>†</sup> Significance level for the effects of genetic group (G), muscle (M), and their interaction (G x M): \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; ns,  $P > 0.05$ . Where the interaction is significant, different superscripts represent significant differences among muscles (x,y; within genetic group) or among genetic groups (a,b; within muscle) at  $P < 0.05$ .

After 72 h storage at 4 °C, the drip loss of fresh meat did not differ among genetic groups or muscles (Table 4.5), but it has to be noted that relatively more LTL samples of purebred Ban pigs exceeded a threshold of 7% drip loss (33% for purebred Ban pigs vs. 8% for PiDu-sired crossbreds). Furthermore, cooking losses at 24 h postmortem did not differ. In contrast, meat from traditionally produced Ban carcasses exhibited a significantly lower ratio of expressible to retained moisture at 24 h postmortem

according to the filter paper press method compared with meat from PiDu-sired crossbreds fattened under semi-extensive production conditions.

**Table 4.5** Water binding properties for muscles (*m. longissimus thoracis et lumborum*, LTL and *m. semimembranosus*, SM) from traditionally raised purebred Ban and pigs from Piétrain x Duroc (PiDu) sires

Trait*	Muscle	Purebred Ban		PiDu-sired crossbreds		RSD	Significance <sup>†</sup>			
		N	LSM	N	LSM		β	G	M	G x M
Drip loss (%)	LTL	30	6.07	11	6.12	1.26	**	ns	ns	ns
	SM	30	5.84	11	6.29					
Cooking loss (%)	LTL	33	30.37	12	30.02	3.72	***	ns	ns	ns
	SM	33	31.28	12	29.58					
FPP (cm <sup>2</sup> /cm <sup>2</sup> )	LTL	33	0.36	12	0.42	0.04	—	***	ns	ns
	SM	33	0.39	12	0.43					

N - Number of observations, LSM - least squares means, RSD - standard deviation of residuals.

\* Drip loss was measured at 72 h post-mortem and cooking loss at 24 h post-mortem. FPP - moisture expressible by the filter paper press method.

<sup>†</sup>Significance level for the effects of sample weight as covariate (β), genetic group (G), muscle (M), and their interaction (G x M): \*\*\*, P < 0.001; \*\*, P < 0.01; ns, P > 0.05.

Although not significant, the intramuscular collagen levels of the LTL muscle of purebred Ban pigs tended to be higher when compared to PiDu-sired barrows (+18%; P < 0.1; Table 4.6).

In Table 4.6, it is also shown that muscles of purebred Ban pigs had considerably lower intramuscular fat levels on a wet tissue basis compared with commercially produced PiDu crossbreds (-51% and -42% relative to LTL and SM of PiDu crossbreds, respectively).

**Table 4.6** Intramuscular fat and collagen concentrations for muscles (*m. longissimus thoracis et lumborum*, LTL and *m. semimembranosus*, SM) from traditionally raised purebred Ban and pigs from Piétrain x Duroc (PiDu) sires

Trait*	Muscle	Purebred Ban		PiDu-sired crossbreds		RSD	Significance <sup>†</sup>		
		N	LSM	N	LSM		G	M	G x M
IMF (%)	LTL	30	1.63	11	3.31	0.90	***	ns	ns
	SM	30	1.80	12	3.08				
IMC (%)	LTL	31	2.50	12	2.04	0.66	ns	—	—

N - Number of observations, LSM - least squares means, RSD - standard deviation of residuals.

\* IMF - Intramuscular fat, IMC - intramuscular collagen.

<sup>†</sup> Significance level for the effects of genetic group (G), muscle (M), and their interaction (G x M): \*\*\*, P < 0.001; ns, P > 0.05.

The composition of lipids of the LTL muscle revealed some remarkable differences between product categories (Table 4.7). Although the small difference encountered in the proportion of stearic acid (C18:0) was significant, the overall proportion of SFA did not differ among genetic groups. The most abundant fatty acid in pork from both sources was oleic acid (C18:1 n-9), which was significantly higher in the LTL of PiDu-sired fatteners, resulting in a considerably higher concentration of MUFA in the loin of the commercial crossbreds. In contrast, linoleic acid (C18:2 n-6) was detected at a significantly higher percentage in the traditional meat product. Consequently, the share of PUFA, the P/S ratio, and also the peroxidizability index, were significantly higher in traditional Ban pork.

**Table 4.7** Lipid composition of muscle (*m. longissimus thoracis et lumborum*) from traditionally raised purebred Ban and pigs from Piétrain x Duroc (PiDu) sires

Trait*	Purebred Ban		PiDu-sired crossbreds		RSD	Significance <sup>†</sup>
	N	LSM	N	LSM		
C16:0 (%)	24	23.50	12	24.01	1.16	ns
C18:0 (%)	24	13.10	12	11.65	1.34	**
C16:1 n-7 (%)	24	1.51	12	2.44	0.64	**
C18:1 n-9 (%)	24	38.49	10	46.89	4.34	***
C18:2 n-6 (%)	24	17.39	11	10.47	4.28	**
∑ SFA (%)	24	38.48	12	38.52	2.10	ns
∑ MUFA (%)	24	41.59	11	49.43	4.70	**
∑ PUFA (%)	24	22.13	11	12.79	6.45	**
P/S ratio	24	0.58	11	0.33	0.18	**
Peroxidizability index	23	29.70	11	17.02	9.03	**

N - Number of observations, LSM - least squares means, RSD - standard deviation of residuals.

\* SFA - Saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids. The P/S ratio was calculated as  $\sum \text{PUFA} / \sum \text{SFA}$  and the peroxidizability index was calculated according to Arakawa and Sagai (1986).

<sup>†</sup> Significance level: \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; ns,  $P > 0.05$ .

## 4.4 Discussion

### Distinguishing carcass characteristics of traditionally produced Ban pigs

The marked differences in carcass yield of Ban pigs when compared to commercial crossbreds is expected and mainly attributable to the effect of sire breeds and the long-term selection on growth and lean meat production in the paternal lines. The Piétrain breed is particularly known for marked muscling and a large loin eye area, even when compared to other modern breeds (Gil et al., 2008). The superior carcass yield of PiDu-sired porkers may have been further enhanced by feeding improved diets. During the study period, the diets of local Ban pigs were mainly based on banana pseudostem and rice bran. Similar diet compositions were reported for traditional pig husbandry systems of other regions of the SEAM (Nakai, 2008; Phengsavanh et al., 2010; Riedel et al., 2012). Banana pseudostem is readily available around the farm premises, but characterized by low percentages of dry matter, crude protein and gross energy. All farmers raising crossbred fatteners

added ground maize grain to the diet, enhancing the dry matter percentage and the gross energy content of the rations. Also vegetable leaves and brewer's grains were important ingredients in most of the preparations for commercial crossbreds, where particularly the brewer's grains represented an important source of dry matter, gross energy and crude protein. Using data from the Animal Feed Resources Information System (2016), it could be estimated that crossbreds consumed 1.8 times more dry matter on a daily basis than Ban pigs. As a result, commercial fatteners could have realized an estimated 2.3 and 2.0 times higher daily intake of crude protein and gross energy. However, no nutritive analyses of the diets have been carried out in this study, and the composition and nutritive value of diets may have varied considerably on household level. Purebred Ban fatteners revealed a reduced dorsal fat layer compared to commercial crosses, whereas, generally, the opposite is found when comparing slow growing breeds with modern genotypes at equal age (e.g., Wood et al., 2004), but this can at least be partly explained by the exceptionally low slaughter weight of Ban pigs. For older Ban pigs, a rapid increase in fat deposits would be expected, as suggested by the results of Hau (2008), where purebred Ban pigs displayed a backfat thickness of 30 mm at an age of approximately 10 months. This indicates that timing of slaughter in relation to maturity is crucial for the success of marketing of specialty Ban carcasses, because for premium markets lean carcasses at lower ages are requested. Fatter Ban pigs can only be sold in rural markets at lower prices per kg of live weight.

### **Distinguishing physico-chemical traits of traditionally produced Ban pork**

Early pH fall and ultimate pH are widely used as indicators for undesired meat conditions such as pale, soft, exudative (PSE) and DFD pork. The rate of pH fall in relation to high pre-rigor temperature in the muscle can have pronounced effects on postmortem metabolism, as well as protein denaturation, and, consequently, on meat quality, and several studies documented breed differences in pH fall and ultimate pH when comparing slow growing and high performing breeds (Chang et al., 2003; Renaudeau and Mouro, 2007). In the present study, the fall of the early postmortem pH was accelerated for PiDu-sired crossbreds compared to Ban pigs, however, it is suggested that these differences in pH development are practically not relevant because pork from PiDu-sired crossbred exhibited no further indications of the PSE condition. Ultimate pH has a marked influence on consumer perception of pork eating quality, but currently no differences in incidence of meat defects between the traditional Ban pork and pork from semi-extensively reared crossbreds are expected. The relative advantage of local Ban pork in terms of the absence of PSE might be more pronounced, if compared to pork from industrially produced completely exotic crossbreds segregating for the halothane gene (Webb and Casey, 2010).

Rather than being a consequence of combined rearing and genetic effects, sporadically occurring high pH values at 24 h postmortem in both product categories could indicate a depletion of glycogen reserves prior to slaughter as a consequence of stress and higher physical activity of the pigs (Henckel et al., 2002). Under the prevalent slaughtering conditions, genetic disposition for reduced meat quality is expected to be rather reflected in an increased incidence of DFD than of PSE. The frequency of LTL muscles exhibiting ultimate pH values > 6.0 amounted to 9% for Ban fatteners and to 17% for the crossbreds. Because of the small sample size, the difference in proportions between the groups was not significant ( $P = 0.598$ ; unpublished), but should be further investigated. The higher pH of DFD meat could promote microbial growth and negatively impact meat safety, particularly under the conditions of informal pork value chains, where a continuous cold chain cannot be safeguarded.

The preference of consumers is substantially influenced by product appearance, especially by its color. However, meat color differences of fresh meat from the two genetic groups were small in this study, and not sufficient for effectively discriminating the product categories. It is questionable whether the differences in instrumental color found when comparing the ham of both genetic groups are detectable by human vision, given that the difference in JCS was non-significant. Similarly, Renaudeau and Mouro (2007) observed no differences in meat color of the *longissimus dorsi* muscle when comparing Caribbean Creole pigs to a commercial breed at a body weight at slaughter of 90 kg, but significantly lower L\* and b\* values for the *semimembranosus* muscle of the slow growing Creole breed. Meat lightness and redness differed particularly when comparing Iberian pig lines to a commercial crossbred (Estévez et al., 2003; Estévez et al., 2006), with lower L\* values and higher a\* values for Iberian pork. Estévez et al. (2003) detected significant breed differences for the heme pigment content of *longissimus dorsi* between Iberian pigs and a commercial crossbred, which may underlie the differences in color. Yet, breed effects on meat color and pigmentation might be exaggerated when slow growing breeds are slaughtered at a higher age, because heme pigment levels and the redness of the meat are supposed to increase with age (Trefan et al., 2013). For the traditionally produced carcasses, a strong color gradient was observed. The differences in meat color between LTL and SM of purebred Ban pigs were backed by differences in pigment content, which could be related to the divergent function and metabolic profiles of muscles. In line with this assumption, Gil et al. (2008) found that oxidative traits, including pigment content, were more pronounced in the *semimembranosus* muscle compared to the *longissimus thoracis* muscle, which had a higher glycolytic activity.

The ability of meat to retain water is associated with purge loss and processing quality, but also with the sensory perception of fresh and cooked meat. Traditionally produced Ban pork did not reveal improved water binding properties when compared to meat from the exotic sired crosses also rather extensively produced in the same region. Caribbean Creole had significantly reduced drip and cooking losses compared to Large White pigs under standardized experimental conditions (Renaudeau and Mouro, 2007). It should be noted that in the previously mentioned study, the effect of slaughter weight was accounted for, but age was a confounding factor. When slaughtered at the same age, Duroc exhibited higher drip losses than traditional British breeds, but Large White revealed the lowest purge loss and did not differ from traditional British breeds (Chang et al., 2003). Drip loss as an indicator for water holding capacity is closely related to the postmortem pH development (Schäfer et al., 2002). The absence of pronounced differences in postmortem pH development between pork from both production contexts in the present study might, thus, partly explain the absence of significant differences in drip and cooking loss. However, factors such as proteolytic activity, connective tissue characteristics, and the amount of intramuscular fat could also contribute to water mobility within meat during storage and water loss during cooking (Pearce et al., 2011). Only in a controlled experiment the water binding potential of Ban meat in relation to pork from conventional genotypes could be determined.

Wheeler et al. (2000) showed that the tenderness scoring of pork muscles is influenced by collagen properties in relation to proteolysis and sarcomere length. In the present study, intramuscular collagen levels tended to increase for traditionally produced LTL from Ban pigs. Instrumentally assessed tenderness between traditional British pig breeds and modern lines differed significantly, with *longissimus dorsi* derived from Duroc being the toughest, followed by Tamworth and Large White samples, which exhibited similar values, and Berkshire, which revealed the most tender meat



(Chang et al., 2003). Whereas Western consumers favor more tender meat (Moeller et al., 2010), no information is available about preferences of Vietnamese consumers, which makes it difficult to create assumptions on acceptability based on intramuscular collagen levels. Additionally, the variation in intramuscular collagen would probably not allow for the discrimination of product categories by sensorial assessment.

## **Distinguishing lipid characteristics of traditionally produced Ban pork**

In contrast to the present findings, most studies comparing slow growing traditional genotypes to modern breeds reported higher intramuscular fat contents for the former (e.g., Estévez et al., 2003; Renaudeau and Mourot, 2007). Wood et al. (2004) pointed towards a more complex situation, with increased marbling fat in Duroc pigs and traditional Berkshire compared to Large White and Tamworth pigs slaughtered at equal age. For intramuscular fat accumulation, additional factors besides breed have to be taken into account. As previously mentioned, the diets of Ban pigs were characterized by a low nutrient density, while for PiDu-crossbreds an insufficient supplementation with essential amino acids could be proposed. Deficiency in essential amino acids is common for homemade diet preparations for pigs on smallholder farms in Vietnam (Pham et al., 2010). Thus, periods of restricted alimentation through a high dilution of nutrients and a low maturity at slaughter age could have specifically contributed to the low intramuscular fat percentage of pork derived from traditionally raised Ban pigs, while a deficit in lysine in diets for PiDu-sired crossbreds could have resulted in their comparatively high intramuscular fat concentration (Da Costa et al., 2004). In line with the results obtained for the commercial crossbreds in the present study, Luc et al. (2014) observed high intramuscular fat levels of 2.8% for commercial crossbred barrows raised under smallholder conditions in northern Vietnam. This value is slightly lower compared to the values for PiDu-sired crossbreds presented herein, which could be due to the higher proportion of exotic blood (25% Mong Cai and 75% exotic) in the crosses investigated by Luc et al. (2014). In Ban pigs, compensatory fat deposition during the later stages of maturation could result in highly marbled meat cuts, as indicated by dramatically increased subcutaneous fat deposits reported by Hau (2008). The impact of the intramuscular fat percentage on eating quality is controversial. Ciobanu et al. (2011) indicated that correlations between consumer acceptability and intramuscular fat level across several studies were relatively high, ranging from 0.54 to 0.68, but Moeller et al. (2010) hardly detected any effect on consumer acceptability below a threshold level of 5 to 6% of intramuscular fat.

The reasons for the remarkable differences in fatty acid profiles between product categories cannot be traced back with certainty because both aspects, genetic and nutritional, contribute to variation in the fatty acid composition. The fatty acid pattern of the traditional product, however, resembles more that of leaner modern pig breeds than slow growing genotypes, whereas the lipid composition of LTL of PiDu crossbreds was closer to that found for muscles of local European purebreds (Estévez et al., 2003; Estévez et al., 2006; Renaudeau and Mourot, 2007). Więcek et al. (2011) showed that feed restriction can affect the fatty acid composition of *longissimus thoracis* and increase the P/S ratio. Under traditional extensive husbandry conditions, frequently occurring fluctuations in feed quantity and quality could, therefore, contribute to the specific fatty acid pattern of the LTL of Ban pigs. Furthermore, it is assumed that the limited development of the fat deposits of Ban pigs could have contributed to the fatty acid composition of the loins of traditionally produced Ban pigs, because dietary PUFA get less 'diluted' by de novo synthesized fatty acids. Variations in lipid quality have implications for the shelf life, technological quality, and also health attributes of pork products, but trade-offs between these domains exist. Since the higher share of PUFA in traditional pork was at the expense of the proportion of MUFA, but not of SFA compared with the commercial product, no major health benefits are expected from the consumption of traditionally produced Ban meat (FAO, 2010). The significantly increased peroxidizability index of the traditional pork product compared to commercial meat, however, indicated a higher number of double bonds in fatty acids of LTL in Ban

pork, which could increase its proneness to oxidation. Therefore, specific attention has to be put on the shelf life of fresh traditional products from Ban pigs, especially when they are transported over long distances. With respect to the processing and storage characteristics of manufactured goods from Ban, the lipid composition of the subcutaneous tissue is of higher importance than the composition of the intramuscular lipids, however the former was not the subject of the present study.

### **Conclusions and implications**

The marketing of pork derived from local pig breeds as a specialty food item could be an important source of additional income for rural smallholders of the SEAM, provided the products are distinguishable from commodity pork. Exemplified for the Vietnamese Ban breed, this study demonstrated that traditionally produced local pork indeed exhibits distinctions compared to pork from crossbreeds produced under semi-extensive conditions. It was shown that, besides smaller cut dimensions and a reduced amount of backfat, particularly features related to lipid characteristics allow for a clear discrimination of traditionally produced local pork. A lower incidence of the DFD defect could not be excluded. These peculiarities could provide the basis for a marketing concept to fill market niches on the increasingly commodified pork markets of Vietnam. The differences are supposed to be largely related to genetic background and its covariance with regional management and feeding practices. These confounding factors aggravate the establishment of causal relationships with respect to the impact of genetic background on meat quality, but are highly relevant for the evaluation of the products under practical conditions. The extent to which these results can be extended to pork products of other SEAM production conditions, including (semi-)intensively produced pork derived from crosses between exotic breeds, has to be further investigated.

Finally, there are still major constraints for designing a value chain for specialty pork that would contribute to the rural development of the Vietnamese uplands and elsewhere in the SEAM. For instance, it remains questionable whether the most disadvantaged producers are capable of meeting the market demand for local pigs in terms of volume, timeliness and quality, and whether they would actually benefit from price premiums in view of high transaction costs in complex value chains. However, the present results may contribute to connecting marginalized farmers from ethnic minorities of the mountainous areas of the SEAM to remunerative urban 'high-end' pork markets in the lowlands, and thereby support the conservation of local Southeast Asian pig genetic resources.

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## **5 Paper IV: Tailoring slaughter weight of indigenous Vietnamese Ban pigs for the requirements of urban high-end niche markets**

P.C. Muth<sup>1</sup>, L.T.T. Huyen<sup>2</sup>, A. Markemann<sup>1</sup>, A. Valle Zárate<sup>1</sup>

<sup>1</sup>Department of Animal Husbandry and Breeding in the Tropics and Subtropics, Hans-Ruthenberg-Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Garbenstr. 17, 70593 Stuttgart, Germany

<sup>2</sup>Department of Economics and Livestock Farming Systems, National Institute of Animal Science, Thuy Phuong, Bac Tu Liem, Hanoi, Vietnam

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### ***Rationale***

Slaughtered at heavy live weights, carcasses of the autochthonous Vietnamese Ban pig breed are not marketable on the highly remunerative niche markets of the urban Red River Delta. Therefore, Ban pigs are traded often at just 10 to 15 kg to avoid excessive fat accumulation. However, already, a moderate increase of slaughter weights might lead to rising profits for smallholder farmers in the uplands.

### ***Objective and scope***

- Thus, this research aimed at investigating the effect of increasing live weight on the carcass composition and meat quality of indigenous Ban pigs qualifying for commerce on high-end markets.
- Fifty-six castrated male Ban fatteners with body weights ranging from 6.5 to 29.3 kg were collected from smallholder farmers applying a feeding system based on banana pseudostem (*Musa* spp.) and slaughtered by local slaughterers according to common practice. The carcass quality of 51 animals and, additionally, meat quality parameters of the loin and ham of 56 animals were assessed.
- For the evaluation of increased live weights on commodity prices, two pricing systems based on actual market prices for Ban fatteners in Son La town and Hanoi were suggested.

### ***Main Findings***

- Carcass fat depots did not significantly increase when body weights were raised from 12 to approximately 20 kg, and the distribution of primal cuts and the meat quality of the loin and ham were relatively stable.
- Thus, the currently applied slaughter weights for purebred Ban pigs appear to be suboptimal, and a moderate increase of slaughter weights to approximately 20 kg is feasible and could result in monetary benefits.
- In this context, the role of small-scale slaughterers for value chain upgrading is discussed.
- In conclusion, increasingly formalized value chains for traditional pork products could provide the potential to not only contribute to rural development in the Southeast Asian Massif, but also to the conservation of animal genetic resources of a highly valuable eco-cultural region.

## 5.1 Introduction

Pork consumers in Vietnam and other Southeast Asian countries increasingly opt for low-fat pork, and substitute cooking lard with vegetable oil, resulting in a rapidly growing commodity market for lean pork (Binh et al., 2007; Borin et al., 2010). In northern Vietnam, this market is predominantly accessed by small-scale producers embedded in a favorable socioeconomic framework. In order to cope with the increased demands and requirements imposed by competitive commodity markets, they shifted to, over the years, crossbreeding with modern pig lines and increased slaughter weights (Luc et al., 2014). Consequently, the yearly output per head of the Vietnamese pig sector continuously rose (FAOSTAT, 2015), and, additionally, lean meat yield improved. In contrast, ethnic minority smallholders in the remote uplands of northwestern Vietnam are barely connected to markets, and practice subsistence-oriented extensive pig husbandry with slow-growing autochthonous breeds (Lemke et al., 2006). For these smallholders, niche markets could provide a viable alternative to the commodity market. Indeed, the Food and Agriculture Organization of the United Nations proposed niche markets as a chance for poor livestock keepers to increase their incomes (FAO, 2011). But, while researchers have analyzed niche pork markets in Europe (Bonneau and Lebret, 2010), particularly those for Iberian pork, and the United States (Honeyman et al., 2006; Hueth et al., 2007), such research within developing countries is lacking. For remote smallholders in northern Vietnam, access to niche pork markets could serve as an additional source of income to crop production, and reduce their dependency on world market prices when selling their cash crops (Keil et al., 2011).

In northern Vietnam, affluent urban consumers in particular show an increasing interest in meat from indigenous pig breeds fed on 'natural' diets (Lapar et al., 2010; Pedregal et al., 2010), indicating that value chains for specialty pork could be extended to urban markets. Smallholders could even exhibit some competitive advantages in supplying niche markets: firstly, remote smallholders keep native pig breeds and, secondly, they apply traditional feeding strategies. Both of these attributes are particularly appreciated by consumers. In the upland region of northwestern Vietnam, smallholders keep the indigenous Ban (synonymous with Meo and Hmong) breed. Under extensive conditions, this pig breed is characterized by a low reproductive performance (7.2 piglets born litter<sup>-1</sup>) and growth rate (65 g daily gain until an age of 180 days) (Lemke et al., 2008). Target markets for Ban pork could be represented by specialty restaurants or 'green' food shops, an organic-like market segment in Hanoi and its surroundings (Le et al., 2016).

Linking rural smallholders embedded in a community-based pig breeding program to these remunerative markets by a specialty food supply chain has been proposed as a sustainable rural development concept in northwestern Vietnam (Herold et al., 2010; Valle Zárate and Markemann, 2010), and could be transferred to other regions of the Southeast Asian Massif where similar conditions prevail, for instance, to the highlands of Laos (Phengsavanh et al., 2010) or southwest China (Neo and Chen, 2009; Riedel et al., 2014). Phuong et al. (2014) showed that restaurant owners in the lowlands of northern Vietnam paid more for pure Ban pork compared to pork from crossbred pigs and exotic purebreds. Additionally, it was found that live weight significantly contributed to price differentiation among purebred Ban hogs. Ban pigs with a live weight lower than 12 kg were priced 4-17% higher by restaurant owners compared to pigs with an average weight of more than 18 kg, which was assumed to be attributable to the increasing fat content of heavier and older pigs [20]. The urban high-end retail sector also puts emphasis on low fat contents of the carcass and, therefore, demands pigs with particularly low live weights (Le et al., 2016). According to Mathias et



al. (2010), understanding and maintaining product quality is crucial in order to demand premium prices in niche markets, whereas not being able to adhere to quality requirements can result in market failure. To our knowledge, data on the carcass quality of pigs slaughtered at low body weights is extremely rare, although products of this type are wide spread (e.g., suckling pigs in Europe, lechón in Latin America and the Philippines, local piglets in Southeast Asia and China). Low weight pigs are often consumed on special occasions, such as private or public festivities (e.g., New Year, harvest festivals) and/or religious celebrations. It should be noted that hogs vary in their genetic origin and therefore reach their market weight at different ages. In China, Southeast Asia and the Philippines, local pigs are the usual preference (Neo and Chen, 2009; Phuong et al., 2014), whereas in Europe, fast growing crossbreeds are slaughtered at a low age to produce suckling pigs. In either case, benchmark data on the carcass configuration of slaughter hogs is required in order to allow for coordination of the value chain, particularly if market acceptability is strongly dependent on carcass quality.

Thus, in order to develop a sustainably competitive marketing grid for specialty native pigs, slaughter weight in relation to carcass tissue composition is of high importance. Elevated slaughter weights could reduce transaction and processing costs per unit of output, which is essential for processors and retailers, and increase the yearly output of meat per sow, which is important for producers. Alterations in carcass composition, for instance, an increase in the amount of subcutaneous fat depots, are often the major limiting factor with regard to increasing slaughter weights in the commodity sector, a principle that also applies to the Ban specialty market. When slaughtered at live weights as commonly applied in rural areas, i.e. at 50 to 60 kg, carcasses of indigenous breeds are not marketable in niche markets, while a moderate increase in live weight at slaughter from, currently, a maximum of 15 kg to around 20 kg could be acceptable and lead to rising profits for stakeholders along the specialty pork value chain in northern Vietnam. Since meat quality is a specific attribute appreciated by consumers of traditional pork in Vietnam (Pedregal et al., 2010), potential variations in the palatability of fresh meat with increasing live weight are also of high interest.

Consequently, the aim of this research was to investigate the effect of increasing live weight on the composition of low weight indigenous Ban carcasses destined for urban high-end markets. One goal was to identify an optimal slaughter weight to produce carcasses with desired attributes as a basis for a marketing grid for vertical coordination between remote smallholder producers and the high-end retail sector. Therefore, Ban pigs were collected under varying environmental conditions in relation to available feed resources and the housing system (pen, enclosure, semi-scavenging) typical for the extensive smallholder pig husbandry system in northern Vietnam and slaughtered by small-scale slaughterers (a capacity of one to two pigs per day) by common practices. Additionally, this research investigated whether meat quality of indigenous Ban pork is affected by changes in live weight.

## **5.2 Material and methods**

### **Animal selection**

This study was carried out from October 2013 to February 2014 in Son La city (21° 19'37" N, 103° 54'51" E) and its surrounding districts. Son La province is a mountainous province in northwestern Vietnam bordering Laos in the south. In total, 56 purebred Ban pigs were selected from different litters, dams and farms. This selection procedure was aimed at drawing a sample representative for slaughter pigs as available on the market, i.e. offspring from unrelated animals raised and finished

under environmental conditions varying in dependence of household resources. Data on the rearing environment of the pigs, including information on feeding, was collected by the use of a simple semi-structured questionnaire (n = 41). Slaughter pigs were castrated male pigs at an age of 5 to 7 months, assigned for marketing at a price premium to restaurants and the high-end food retail sector in the urbanized Red River Delta.

### **Slaughter and carcass quality measurements**

Animals were slaughtered in Son La city according to common slaughtering practice, i.e. bleeding without prior stunning, by three slaughterers representing typical small-scale slaughter locations (a capacity of one to two pigs per day). To date, no larger abattoirs exist in Son La province. A maximum of four pigs per day was slaughtered on 19 slaughter days. Pigs were collected the day before slaughter and fasted for at least 12 h prior to slaughter. After bleeding, pigs were scalded by pouring hot water over the body and dehaired manually. Subsequent to evisceration, head, feet and leaf fat were removed. The hot carcass without viscera, head, feet and leaf fat was weighed and the hot dressing percentage calculated relative to the live weight of the fasted animal. The carcass was then split along the backbone and backfat thickness was recorded at the level of the last rib (BF1) and above *gluteus medius* (BF2). The carcass length was measured as the shortest distance from the first thoracic vertebra to the pubic symphysis. The loin eye area (LEA) was recorded on the cross-section of the loin between thoracic vertebrae 13 and 14. Afterwards, the right carcass half was weighed and dissected, according to Nissen et al. (2006), into the most valuable primal cuts (i.e. shoulder, loin and tenderloin, leg). Primal cut yields were expressed relative to the hot carcass weight. Primal cuts and the belly were then dissected further into the soft tissues (lean, fat, skin) and bone. The carcass components were weighed and tissue composition expressed relative to the hot carcass weight. Samples of the loin (*longissimus thoracis et lumborum*, LTL) and ham (*semimembranosus*, SM) were excised, and partly stored refrigerated at 4°C for meat quality analysis or frozen at -18°C for biochemical analysis.

### **Meat quality determination**

The pH values at 45 min (pH1) and 24 h (pH24) postmortem of the LTL and SM muscles were measured using a handheld pH meter (pH-STAR, Matthaeus GmbH & Co. KG, Nobitz, Germany), calibrated at pH 4.6 and 7.0, respectively.

The Japanese Color Score (JCS) was determined subjectively at 24 h postmortem on the cross-section of the LTL and SM, using a scale ranging from 1 (extremely pale) to 6 (extremely dark red). Meat color (CIE L\*, a\* and b\*) was determined at the same location by a colorimeter (model CR-400, Konica Minolta Inc., Tokyo, Japan) with a 2° observer angle and illuminant C setting. Eight readings were averaged for analysis.

Drip loss (DL) in LTL and SM, respectively, was calculated as weight loss after storage at 4°C until 72 h postmortem relative to the initial sample weight. Cooking loss (CL) at 24 h postmortem of LTL and SM, respectively, was defined as the weight difference after cooking to an internal temperature of 85°C relative to the sample weight before cooking. For the determination of water holding capacity (WHC), small meat samples (ca. 0.3 g) were placed onto filter paper and pressed between two plexiglass blocks for 5 min. Higher ratios of the meat area over the area of expressible fluid indicated an increased WHC. The procedure was repeated three times for LTL and SM, respectively.

Water soluble myoglobin (Mb) content was determined according to Faustman and Phillips (2001), with slight modifications. In brief, LTL and SM samples ( $5 \pm 0.02$  g), respectively, were minced, mixed with 20 ml of bidest. water, homogenized and then centrifuged. The supernatant was then filtered by vacuum filtration through a Whatman<sup>TM</sup> microfiber filter grade GF/C (GE Healthcare, Little Chalfont, UK) and centrifuged at  $22,000 \times g$  for 25 min. From the absorbance at 525 and 730 nm, the concentration of Mb in mg per g wet tissue was calculated following Van Laack et al. (1996), but without accounting for filtration losses.

Intramuscular fat (IMF) content in LTL and SM samples ( $3 \pm 0.1$  g), respectively, was determined by the Department of Feed and Product Analyses, National Institute of Animal Sciences in Hanoi, Vietnam, using a 1045/1046 Soxtec<sup>TM</sup> extraction system (FOSS Analytical AB, Hoeganaes, Sweden). The concentration of IMF was determined gravimetrically and expressed relative to the wet sample weight.

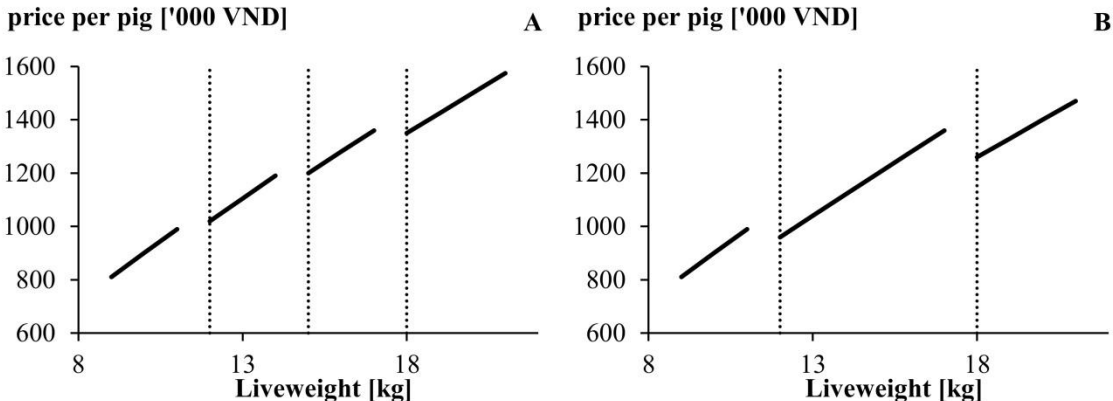
### Statistical analysis

For the statistical analysis, the individual pig was considered as an experimental unit. A mixed model (1) was fitted for the evaluation of the effect of live weight on carcass measurements, carcass tissue composition, and weight and distribution of primal cuts of Vietnamese Ban pigs using the PROC MIXED function of SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

**Table 5.1** Nominal classification of male castrated Ban pigs into live weight categories

Live weight category	N	Live weight at slaughter in kg		Age at slaughter in months	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
<12 kg	14	9.8 $\pm$ 1.5	6.5 – 11.8	6.1 $\pm$ 0.4	5.4 – 6.7
$\geq$ 12 to <15 kg	13	13.0 $\pm$ 0.7	12.0 – 14.5	6.3 $\pm$ 0.4	5.9 – 6.8
$\geq$ 15 to <18 kg	15	16.0 $\pm$ 0.7	15.0 – 17.0	6.3 $\pm$ 0.2	5.8 – 6.4
$\geq$ 18 kg	9	20.3 $\pm$ 1.9	18.0 – 22.5	6.6 $\pm$ 0.3	6.2 – 6.9

To obtain balanced class ranges, the data set was limited to 51 observations distributed over four live weight categories (<12 kg; ≥12 to <15 kg; ≥15 to <18 kg; ≥18 kg; Table 5.1), representing a fixed effect in the model. The live weight deviation of an individual pig from its respective category mean was added as an intragroup regression term. The age of the pig was not included as a covariate, because the exact age in days at slaughter was not available for each pig. Because of the variation in the weather between the slaughter days and in the handling, killing, eviscerating, and dissecting of the pigs between the small-scale abattoirs, slaughter day (19 levels) nested within the slaughter location (three levels) was considered a random effect.



**Figure 5.1** Farm gate prices in Vietnamese Dong (VND) per Ban pig based on two degressive pricing systems (in '000 Dong per kg of live weight for system A: <12kg: 90/kg; ≥12kg to <15kg: 85/kg; ≥15kg to <18kg: 80/kg; ≥18kg: 75/kg; and system B: <12kg: 90/kg; ≥12kg to <15kg: 80/kg; ≥15kg to <18kg: 80/kg; ≥18kg: 70/kg); dotted vertical lines represent thresholds in live weight above which per kg prices decrease.

For the evaluation of the effect of live weight on commodity prices, two pricing systems were suggested, both assuming declining per kg prices with increasing live weights (Figure 5.1). The prices were derived from actual per kg live weight market prices for Ban fatteners in Son La and Hanoi (Le et al., 2016). The commodity price was calculated by dividing farm gate prices of pigs by the weight of the eviscerated carcass and lean meat, respectively. Model (1) reads as follows:

$$y_{ijkl} = \mu + LC_i + \beta_i L_{ijkl} + \text{day}(\text{location})_{jk} + e_{ijkl} \quad (1)$$

where  $y_{ijkl}$  = the dependent variable;  $\mu$  = the overall mean;  $LC_i$  = the fixed effect of the  $i$ th live weight category ( $i = 1, 2, 3, 4$ );  $L_{ijkl}$  = the deviation of the individual live weight from the mean weight of the respective live weight category;  $\beta_i$  = the regression coefficient for  $L_{ijkl}$  within the  $i$ th live weight category;  $\text{day}(\text{location})_{jk}$  = the random effect for the  $j$ th slaughter day ( $j = 1, \dots, 19$ ) nested within the  $k$ th slaughter location ( $k = 1, 2, 3$ ); and  $e_{ijkl}$  = the error term. Errors were assumed to be normally distributed and the variance of errors as constant across observations. For a multiple pairwise comparison of the least squares means among live weight categories, the PDIFF statement was applied using the Tukey-Kramer adjustment (ADJUST = tukey) for unbalanced data. The level of significance was set to  $\alpha = 5\%$ .

For the evaluation of the effect of live weight on meat quality traits, all 56 observations on Ban finisher pigs representing a live weight range from 6.5 to 29.3 kg were considered. Mixed regression using PROC MIXED was conducted separately for LTL and SM muscles. Linear and quadratic regression terms on live weight were entered stepwise, starting from an unconditional model (2) which only accounted for the random effect. The full model (3) included a general intercept and a linear and quadratic term for live weight as well as an error term. The models are denoted as follows:

$$y_{ijk} = \mu + \text{day}(\text{location})_{ij} + e_{ijk} \quad (2)$$

$$y_{ijk} = \mu + \beta_1 L_{ijk} + \beta_2 L_{ijk}^2 + \text{day}(\text{location})_{ij} + e_{ijk} \quad (3)$$

where  $y_{ijk}$  = the dependent variable;  $\mu$  = the overall mean;  $L_{ijk}$  = live weight and its regression coefficient  $\beta_1$ ;  $L_{ijk}^2$  = square of live weight and its regression coefficient  $\beta_2$ ;  $\text{day}(\text{location})_{ij}$  = the random effect for the  $i$ th slaughter day ( $i = 1, \dots, 19$ ) nested within the  $j$ th slaughter location ( $j = 1, 2, 3$ ); and  $e_{ijk}$  = the error term. The accuracy of models was comparatively evaluated based on the Akaike information criterion (AIC) and the Bayesian information criterion (BIC). Only when the fit of the model was improved were additional terms to the general intercept included. Errors were assumed to be normally distributed and the variance of errors as constant across observations.

### 5.3 Results

Fifty-six percent of the Ban pigs were permanently housed in improvised pens, 15% were kept in spacious enclosures and 29% were left to roam freely during daytime. All of the pigs were fed restrictively on cooked rations during the fattening phase (weaning to slaughter). In total, 93% of the farmers who responded to the questionnaire used banana pseudostem (*Musa ssp.*) as a feed ingredient, followed by rice bran (83%), green leaves (39%) and finely ground maize (37%). Usually, more than half of the approximately 4 kg of fresh matter in one pig's daily ration was composed of banana pseudostem.

As expected, the carcass weight and carcass length of Ban pigs slaughtered at weights from 6.5 to 22.5 kg were closely related to the live weight and continuously increased over live weight categories

(Table 5.2). Loin eye area was significantly higher for Ban pigs weighing  $\geq 15$  kg compared to those in the lower weight categories. Surprisingly, the live weight category was a poor predictor for backfat thickness measured at the last rib (BF1,  $p = 0.093$ ). BF2 was significantly reduced comparing live weight category  $< 12$  kg to both intermediate groups, weighing  $\geq 12$  to  $< 18$  kg. Hot dressing percentage was significantly lower (-5.3 percent points) for pigs in the lowest weight category ( $< 12$  kg) compared to those ranging from  $\geq 15$  to  $< 18$  kg live weight. However, the hot dressing percentage of pigs weighing  $< 12$  kg was also reduced by more than 3 percent points compared to the categories of hogs weighing  $\geq 12$  to  $< 15$  kg and  $> 18$  kg, respectively.

**Table 5.2** Least squares means (LSM) for slaughter characteristics and carcass measurements for different live weight categories of male castrated Ban pigs.

Trait	Live weight category								RSD <sup>a</sup>	Sig. <sup>b</sup>
	<12 kg		$\geq 12$ to <15 kg		$\geq 15$ to <18 kg		$\geq 18$ kg			
	N	LSM	N	LSM	N	LSM	N	LSM		
Carcass weight in kg	14	5.1 <sup>a</sup>	12	7.2 <sup>b</sup>	15	9.1 <sup>c</sup>	9	11.1 <sup>d</sup>	0.4	<0.001
Carcass length in cm	14	35.4 <sup>a</sup>	12	37.5 <sup>b</sup>	15	39.4 <sup>c</sup>	9	43.2 <sup>d</sup>	1.2	<0.001
Loin eye area in cm <sup>2</sup>	14	5.6 <sup>a</sup>	13	6.7 <sup>a</sup>	15	8.5 <sup>b</sup>	9	8.3 <sup>b</sup>	1.2	<0.001
Backfat last rib in mm	14	4.2	13	6.0	15	7.8	9	7.6	3.3	0.093
Backfat GM <sup>c</sup> in mm	14	2.6 <sup>a</sup>	13	7.1 <sup>b</sup>	15	6.8 <sup>b</sup>	9	6.6 <sup>ab</sup>	2.4	0.009
Hot dressing in %	14	51.6 <sup>a</sup>	12	55.5 <sup>ab</sup>	15	56.9 <sup>b</sup>	9	54.9 <sup>ab</sup>	3.1	0.012

<sup>a,b,c,d</sup> Within a row, least squares means with common letter are not significantly different at  $\alpha = 5\%$ .

<sup>a</sup> RSD = residual standard deviation.

<sup>b</sup> Sig. = significance of the effect of live weight category.

<sup>c</sup> GM = *gluteus medius*.

Although the live weight categories were narrowly defined, the absolute weight of lean meat in Ban carcasses increased significantly over live weight categories (Table 5.3). Thereby, lean weight increased on average by 30% from one live weight category to the next heavier one. In contrast, carcass fat (subcutaneous and intermuscular fat) only differed significantly when comparing the live weight category  $< 12$  kg to the other categories, which corresponds to the results found for backfat measurements. Skin tissue weights also significantly increased, but less pronounced than with lean tissue. Overall, the quantity of soft tissues significantly increased across live weight categories. A moderate increase over live weight categories was observed for the weight of osseous tissue; the relative per category increase was on average 21%.

Although the absolute amount of lean in the carcass of indigenous Ban pigs continuously increased over live weight categories, there was no difference among live weight groups in relative lean content of the carcasses ( $p = 0.143$ ; Table 5.3). The percentage of subcutaneous and intermuscular fat was significantly increased for the live weight groups heavier than 12 kg compared to the lowest live weight category, but no significant differences were observed among the three heavier weight categories. The model for fat percentage resulted in the highest residual standard deviation (RSD = 5.6), indicating relatively high variation between individual animals for this trait. The relative share of skin and bone tissues in the carcasses slightly decreased over weight categories. Consequently, the lean to bone ratio was significantly wider for the categories  $\geq 15$  kg live weight compared to pigs weighing less than 12 kg.

**Table 5.3** Least squares means (LSM) for carcass tissue weights (right carcass half) and distribution for different live weight categories of male castrated Ban pigs

Trait	Live weight category								RSD <sup>a</sup>	Sig. <sup>b</sup>
	<12 kg		≥12 to <15 kg		≥15 to <18 kg		≥18 kg			
	N	LSM	N	LSM	N	LSM	N	LSM		
<b>Weight in kg</b>										
Soft tissue	14	1.7 <sup>a</sup>	13	2.6 <sup>b</sup>	15	3.2 <sup>c</sup>	9	4.1 <sup>d</sup>	0.2	<0.001
Lean	14	1.1 <sup>a</sup>	13	1.4 <sup>b</sup>	15	1.9 <sup>c</sup>	9	2.4 <sup>d</sup>	0.2	<0.001
Fat	14	0.1 <sup>a</sup>	13	0.5 <sup>b</sup>	15	0.7 <sup>b</sup>	9	0.8 <sup>b</sup>	0.2	<0.001
Skin	14	0.5 <sup>a</sup>	13	0.6 <sup>b</sup>	15	0.7 <sup>b</sup>	9	0.9 <sup>c</sup>	0.1	<0.001
Bone	14	0.8 <sup>a</sup>	13	0.9 <sup>a</sup>	15	1.1 <sup>b</sup>	9	1.4 <sup>c</sup>	0.1	<0.001
<b>Composition in %</b>										
Soft tissue	14	66.3 <sup>a</sup>	12	70.3 <sup>ab</sup>	15	70.6 <sup>ab</sup>	9	73.4 <sup>b</sup>	4.1	0.031
Lean	14	42.1	12	38.4	15	41.3	9	43.5	3.4	0.143
Fat	14	5.0 <sup>a</sup>	12	13.6 <sup>b</sup>	15	14.4 <sup>b</sup>	9	14.0 <sup>b</sup>	5.6	0.007
Skin	14	19.3 <sup>a</sup>	12	18.3 <sup>ab</sup>	15	14.6 <sup>b</sup>	9	15.9 <sup>ab</sup>	3.2	0.007
Bone	14	30.5 <sup>a</sup>	12	26.1 <sup>ab</sup>	15	24.7 <sup>b</sup>	9	24.4 <sup>b</sup>	3.2	0.003
<b>Lean to bone ratio</b>	14	1.4 <sup>a</sup>	13	1.5 <sup>ab</sup>	15	1.7 <sup>b</sup>	9	1.8 <sup>b</sup>	0.2	0.005

<sup>a,b,c,d</sup> Within a row, least squares means with common letter are not significantly different at  $\alpha = 5\%$ .

<sup>a</sup> RSD = residual standard deviation.

<sup>b</sup> Sig. = significance of the effect of live weight category.

In spite of their degressive setup, both exemplary applied pricing systems would provide incentives for producers to increase slaughter weights of Ban hogs (Figure 5.1). But assuming pricing system A (Table 5.4), increasing slaughter weight to more than 15 kg also resulted in significantly lower commodity prices per kg of salable carcass and lean meat for traders, processors and retailers. Furthermore, significantly reduced commodity prices were observed when increasing slaughter weights to more than 18 kg when pricing system B was assumed.

**Table 5.4** Least squares means (LSM) for commodity prices (in '000 Vietnamese Dong per kg) for different live weight categories of male castrated Ban pigs

Pricing system	Commodity							
	Carcass				Lean meat			
	A		B		A		B	
Live weight category	N	LSM	N	LSM	N	LSM	N	LSM
<12 kg	13	173 <sup>x</sup>	13	173 <sup>x</sup>	14	421 <sup>x</sup>	14	422 <sup>x</sup>
≥12 to <15 kg	12	154 <sup>y</sup>	12	145 <sup>y</sup>	13	410 <sup>x</sup>	13	387 <sup>xy</sup>
≥15 to <18 kg	15	141 <sup>z</sup>	15	141 <sup>yz</sup>	15	348 <sup>y</sup>	15	348 <sup>yz</sup>
≥18 kg	9	137 <sup>z</sup>	9	128 <sup>z</sup>	9	314 <sup>y</sup>	9	293 <sup>z</sup>
RSD <sup>b</sup>		9		9		34		33
Sig. <sup>c</sup>		<0.001		<0.001		<0.001		<0.001

<sup>x,y,z</sup> Within a column, least squares means with common letter are not significantly different at  $\alpha = 5\%$ .

<sup>a</sup> Commodity prices were calculated assuming two different degressive pricing systems with producer prices in '000 Vietnamese Dong (VND) per kg live weight; Pricing system A: <12kg: 90/kg; ≥12kg to <15kg: 85/kg; ≥15kg to <18kg: 80/kg; ≥18kg: 75/kg; Pricing system B: <12kg: 90/kg; ≥12kg to <15kg: 80/kg; ≥15kg to <18kg: 80/kg; ≥18kg: 70/kg.

<sup>b</sup> RSD = residual standard deviation.

<sup>c</sup> Sig. = significance of the effect of live weight category.

The variation in absolute weight of primal cuts in Ban carcasses was adequately explained by live weight categories as indicated by low residual standard deviations (RSD ~ 0.1) (Table 5.5). Shoulder, loin and tenderloin as well as leg weights increased significantly across live weight categories by, on average, 26%, 32% and 24%, respectively. In contrast, yields in primal cuts were less affected by live weight category. Only the proportion of shoulder in Ban carcasses decreased slightly, but significantly ( $p = 0.014$ ), by 3 percent points, comparing the lightest weight category to the category of ≥15 to <18 kg.

**Table 5.5** Least squares means (LSM) for primal cut weight (right carcass half) and distribution for different live weight categories of male castrated Ban pigs.

Trait	Live weight category								RSD <sup>a</sup>	Sig. <sup>b</sup>
	<12 kg		≥12 to <15 kg		≥15 to <18 kg		≥18 kg			
	N	LSM	N	LSM	N	LSM	N	LSM		
<b>Weight in kg</b>										
Shoulder	14	1.1 <sup>a</sup>	13	1.5 <sup>b</sup>	15	1.8 <sup>c</sup>	9	2.2 <sup>d</sup>	0.1	<0.001
Loin + tenderloin	14	0.5 <sup>a</sup>	13	0.7 <sup>b</sup>	15	0.9 <sup>c</sup>	9	1.2 <sup>d</sup>	0.1	<0.001
Leg	14	0.7 <sup>a</sup>	13	0.9 <sup>b</sup>	15	1.2 <sup>c</sup>	9	1.4 <sup>d</sup>	0.1	<0.001
<b>Distribution in %</b>										
Shoulder	14	42.9 <sup>a</sup>	12	41.5 <sup>ab</sup>	15	39.9 <sup>b</sup>	9	40.3 <sup>ab</sup>	1.3	0.014
Loin + tenderloin	13	19.2	12	20.0	15	20.0	9	20.9	1.5	0.453
Leg	14	26.5	12	25.2	15	26.6	9	25.3	2.1	0.399

<sup>a,b,c,d</sup> Within a row, least squares means with common letter are not significantly different at  $\alpha = 5\%$ .

<sup>a</sup> RSD = residual standard deviation.

<sup>b</sup> Sig. = significance of the effect of live weight category.

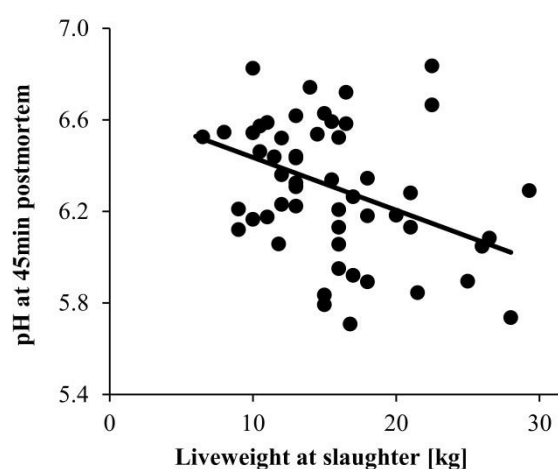


Meat quality parameters of Ban loins and hams were evaluated irrespective of weight and trade classes. For the SM muscle, the unconditional model always provided the better fit based on AIC and BIC values compared to models comprising linear and quadratic regression terms. This indicates that for the live weight range from 6.5 to 29.3 kg, meat quality of the ham, including pH, meat color, pigmentation, water binding capacity and IMF content, was not affected by live weight. In contrast, meat quality of the loin changed with increasing live weight. Per kg increase in live weight, pH measured at 45 min postmortem in the LTL decreased by 0.02 units in a linear manner (Figure 5.2,  $p = 0.005$ ). Also, the water holding capacity of the LTL tended to be affected by live weight (Table 5.6): the final model for drip loss at 72 h postmortem, which provided the best fit according to the AIC and BIC criteria, included a linear ( $p < 0.001$ ) and a quadratic term ( $p < 0.001$ ), while for cooking loss at 24 h postmortem only the linear term tended to be significant ( $p = 0.075$ ) and was, therefore, included in the final model. For cooking loss, the variance component for slaughter day within location/slaughterer was zero, whereas for drip loss 30% of the variance not attributable to slaughter weight was explained by slaughter day within location/slaughterer variation. Variation of meat color and Mb content in the loin was not related to increases in live weight. In agreement with the limited impact of increases in slaughter weight on (subcutaneous) carcass fat deposition, no change in IMF content in LTL was observed for Ban pigs weighing up to 29.3 kg.

**Table 5.6** Coefficients (standard errors in parentheses) for the linear and quadratic dependence of loin meat quality traits on live weight of male castrated Ban pigs and covariance components for the random effects

Trait	Intercept	Linear term		Quadratic term		Covariance	
		$\beta_1$ (SE)	Sig. <sup>a</sup>	$\beta_2$ (SE)	Sig. <sup>a</sup>	Slaughter	Residual
pH at 45 min	6.67 (0.13)	-0.023 (0.008)	0.005			0.005	0.077
Cooking loss in %	26.59 (1.62)	0.179 (0.099)	0.075			0	13.747
Drip loss in %	15.78 (1.83)	-0.990 (0.219)	<0.001	0.024 (0.006)	<0.001	1.069	2.025

<sup>a</sup> Sig. = significance of the linear and quadratic regression terms.



**Figure 5.2** Linear dependence of pH of longissimus thoracis et lumborum measured at 45min postmortem on live weight of male castrated Ban pigs.

## 5.4 Discussion

Data on slaughter performance of pigs at low body weights, as reported for the Vietnamese Ban breed herein, are rare. Under most market conditions, slaughtering finisher pigs at a much higher body weight is economically reasonable and the sale of low weight pigs is regarded as an ancillary activity. However, smallholders in particular could benefit from improved marketing of such niche products. The high-end retail sector of the urban Red River Delta in northern Vietnam exclusively requests slaughter weights of 10 to 15 kg for native pigs, mainly to avoid excessive accumulation of fatness in the final products (Phuong et al., 2014; Le et al., 2016). Yet, moderately increasing slaughter weights could be beneficial for both, producers and downstream stakeholders, but this depends on carcass and meat quality, as well as on aspects of value chain coordination.

### 5.4.1 Discussion of empirical findings

#### Impact of live weight on carcass measurements

The relationships between carcass measurements, such as carcass weight, carcass length and LEA, and body weight in Ban pigs resembled those observed for common three-way crosses and commercial hybrids slaughtered at body weights of >100 kg (Cisneros et al., 1996; Piao et al., 2004; Correa et al., 2006). For Lacombe pigs, Martin et al. (1980) noted that carcass length and the longissimus dorsi area concomitantly increased with slaughter weights from 73 to 137 kg. Covering a range of live weights from 10 to 150 kg, Nieto et al. (2013) showed that for slow growing Iberian pigs, carcass length increased with live weight. From the low allometric growth coefficients obtained for carcass length and the longissimus dorsi area of different genotypes weighing 59 to 127 kg at slaughter, Gu et al. (1992) concluded that these traits are early growth traits. In a study by Hau (2008), Ban pigs were slaughtered at 2.5 times higher body weights (approximately 50 kg) compared to the heaviest live weight category in the present study. Relating his results to the present data suggests that a marked increase in meatiness can still be realized (2.8 times larger LEA for heavier Ban pigs), but increases in carcass length relative to live weight are lower. Thus, as is true for modern breeds slaughtered at commercial body weights, an increase in slaughter weight of indigenous Ban pigs could positively influence economically important carcass traits such as dressing percentage and meatiness. Lemke et al. (2006) observed that the weaning of Ban piglets was performed at an age of two months and a body weight of 4 to 5 kg. The average daily gain before and after weaning until the age of six months was comparable, indicating a low but continuous increase in the body weight of Ban pigs. At least during this phase, the trajectory of most of the carcass measurements of Ban fatteners is similar to that of heavier commercial pigs. In contrast to our results, most studies, except for Piao et al. (2004), reported a significant increase of backfat thickness recorded at the last rib with an increased body or carcass weight of pigs (Gu et al., 1992; Cisneros et al., 1996; García-Macías et al., 1996; Beattie et al., 1999; Wiseman et al., 2007; Choi et al., 2013), which might be attributed to the more intensive feeding regimen applied. Nieto et al. (2013) noted that for Iberian pigs the amount of subcutaneous fat increased at low body weights from only 10 to 50 kg. The same was found for fast growing Piétrain-sired progeny, in which subcutaneous fat accumulation was already initiated at low body weights (Landgraf et al., 2006). Landgraf et al. (2006) also observed a substantial between-animal variation in backfat weight and yield. The absence of a marked effect of increased live weight at slaughter on backfat thickness for Ban pigs could be explained in two ways. Firstly, taking the complex and nonlinear (quadratic) relationship between carcass weight and backfat thickness into account, as demonstrated in a meta-analysis by Trefan et al. (2013), subcutaneous fat deposition in Ban pigs may not have been fully initiated within the limited live

weight span considered in the present study. This could be compensated by excessive subcutaneous fat deposition during later growth periods, as suggested by Hau (2008), who recorded a backfat thickness of 30 mm for Ban pigs slaughtered at approximately 50 kg. The considerable expression of the dorsal fat layer is a major reason for the rejection of heavy Ban pigs by the high-end retail sector. Čandek-Potokar et al. (1998) showed that under circumstances of nutrient deficiency, low backfat levels were maintained. Therefore, we secondly assume that, besides direct genetic effects, environmental conditions might also have affected backfat levels. Below a live weight of 12 kg, hardly any subcutaneous backfat was deposited in Ban pigs, but it has also been found that loin cuts are clearly underdeveloped and dressing percentages unfavorable at these low weights.

### **Impact of live weight on the weight and distribution of tissues and primal cuts**

Results on carcass dissection, obtained from faster growing genotypes reared under more intensive husbandry and feeding conditions compared to the present study, are not in contradiction to the results drawn from the dissection of Ban carcasses. At 20 kg live weight, offspring sired by Piétrain boars exhibited 3.1 kg lean tissue, 0.9 kg adipose tissue and 1.9 kg bones in their carcasses (Landgraf et al., 2006), which is only slightly more when compared to Ban pigs of the same weight (Table 5.3) and linked to the increased carcass weight of modern crosses. The weight of all carcass components of the Piétrain-sired pigs increased until reaching a final weight of 140 kg. Both fat and lean tissue revealed allometric growth coefficients greater than the one relative to empty body weight, while for bone tissue the allometric growth coefficient was lower than one indicative of an early maturing tissue (Landgraf et al., 2006). Gu et al. (1992) noted that for various genotypes slaughtered between 59 and 127 kg the regression of live weight on lean, skin and bone was basically linear, while fat accretion followed a curvilinear trend, i.e. absolute growth of lipid tissues rapidly accelerated during later growth stages. For carcasses from Lacombe pigs with body weights ranging from 73 to 137 kg, Martin et al. (1980) reported that lean and fat weight increased linearly at a similar rate, whereas bone weight increased at a slower rate. Linear trends for absolute growth in lean, and at a slower rate also for bone tissue, were also observed for lightweight Ban hogs from below 10 to approximately 20 kg. When assuming a curvilinear behavior for absolute growth of extramuscular fat depots, as suggested by Gu et al. (1992), it can be assumed that the body weight after which the growth of lipid tissue accelerates has not been reached by most Ban pigs at approximately 20 kg.

When compared to fast growing Piétrain-sired crosses at comparable body weights of about 20 kg (Landgraf et al., 2006), Ban pigs revealed similar proportions of lean (both genotypes approximately 44%), fat (16% for Ban pigs versus 13% for Piétrain-sired pigs) and bone (24% for Ban pigs vs. 27% for Piétrain-sired pigs) in the carcass. Up to a slaughter weight of 60 kg, lean tissue yields in Piétrain-sired progeny increased to 50% and fat tissue yields remained constant at 11%. For Ban pigs weighing approximately 50 kg, however, lean content decreased to 35% and fat content dramatically increased to 41% (Hau, 2008). Thus, the development of proportions of carcass components in Vietnamese Ban pigs probably follows a rather similar pattern to that observed for slow growing Iberian pigs. For this autochthonous breed, Nieto et al. (2013) found that lean yield in shoulder and ham increased when comparing pigs weighing 10 to 25 kg with pigs weighing 15 to 50 kg, but dramatically decreased at higher weights. At slaughter weights of 50 to 100 kg, the proportion of fat in shoulder and ham of Iberian pigs exceeded 35%. Under the extensive husbandry system and a live weight span of up to approximately 20 kg, subcutaneous and intermuscular fat accumulation for Ban pigs does not necessarily have to be controlled by setting lower thresholds on slaughter weight. This could be a consequence of the restrictive feeding practices and the composition of the rations, resulting from

limited household resources, especially during the winter season when the present study took place. It is well-established that lipid deposition in growing pigs does not accelerate until the feeding level exceeds the maximum nutrient intake required for lean deposition (Clutter, 2011). Therefore, restricted feeding and nutrient deficient rations based on banana pseudostem, which are also common in smallholder pig production systems in the uplands of Central Vietnam (Pham et al., 2010) and southwest China (Riedel et al., 2014), possibly contributed to the relatively low share of fat in the carcasses of Ban pigs in the present study. A number of other studies indicate that in fast growing genotypes, lean yield decreases after reaching a slaughter weight of around 90 kg (Martin et al., 1980; García-Macías et al., 1996; Beattie et al., 1999). However, Martin et al. (1980) noted that linear regression of the body weight of Lacombe barrows on lean yield could not be fitted, which is similar to our observations. With increasing body weight, skin and bone yield were reduced, which results from the slow absolute growth rates of these tissues relative to carcass weight or body weight at slaughter (Gu et al., 1992; Landgraf et al., 2006). In summary, no adverse changes in carcass composition were noted for Ban pigs slaughtered at live weights ranging from 12 kg to live weights slightly exceeding 20 kg, while at <12 kg, carcasses contained less fat but more osseous tissue. For higher live weights, lean and fat yields remained stable, but, as demonstrated here, tissue weights, in particular lean weight, further increased with live weight. Additional research is needed for the growth phase between 20 and 50 kg when, according to our assumptions, the cutout will successively deteriorate due to excessive fat accumulation. Also, experiments with defined feeding regimen corresponding to smallholders' practices under seasonally varying conditions are required.

For Ban pigs from 10 to approximately 20 kg, changes in the distributions of primal cuts were marginal and not of practical relevance. Nieto et al. (2013) found low allometric growth coefficients for primal cut weights (ham, shoulder, loin) in Iberian pigs, indicating that they are early growth traits. From 50 kg live weight and above, the cutout percentages of loin, shoulder and ham were reduced, whereas belly yield was increased (Nieto et al., 2013). For fast growing pig lines slaughtered at heavy live weights, increases in primal cut weights were more or less proportional to increases in body or carcass weight (Martin et al., 1980; García-Macías et al., 1996; Cisneros et al., 1996; Latorre et al., 2004; Correa et al., 2006), and, in contrast to Nieto et al. (2013), exhibited allometric growth coefficients close to or even greater than one (Gu et al., 1992; Landgraf et al., 2006). Primal cut distribution was marginally affected by increasing the slaughter weights of the modern genotypes compared, but some alterations in yields have been observed (Martin et al., 1980; Cisneros et al., 1996; Latorre et al., 2004; Correa et al., 2006; Landgraf et al., 2006). These results were partly contradictory between studies; some of the differences may be due to variation in genotype and weight range, and to varying cutting styles. Taking the development of primal cut yields for other genotypes and the results of Hau (2008) for heavy Ban pigs into account, we would expect a further decrease in shoulder yield for heavier Ban carcasses, while loin and particularly belly percentage could increase during the growth period beyond 20 kg live weight.

### **Impact of live weight on meat quality characteristics**

Meat quality parameters are of less relevance for the trading and pricing of Ban pigs compared to carcass characteristics, but they are and will be, nonetheless, crucial with respect to the competitiveness of the product on the pork market. It appears that the pH fall in loin is accelerated for heavier Ban pigs, which could be associated with a concomitant increase in cooking loss. On the other hand, drip loss of loin samples decreased from a live weight of approximately 5 to 15 kg, but remained stable afterwards. According to a recent meta-analysis by Trefan et al. (2013) covering a

range of cold carcass weights from 30 to 150 kg, some meat quality traits revealed a significant dependence on carcass weight, implying that a defined meat quality can be produced by adjusting slaughter weights. Most notably, meat color in the *longissimus* muscle and ultimate pH in the *semimembranosus* muscle were affected by carcass weight, whereas, in contrast to the present study, early pH fall in the *longissimus* was not altered. While marbling scores followed a curvilinear pattern, Trefan et al. (2013) suggested that IMF content was not associated with carcass weight. In contrast to these results, Cisneros et al. (1996) reported linearly decreasing ultimate pH values of the *longissimus lumborum* for barrows and gilts slaughtered between 100 and 160 kg, whereas the IMF percentage linearly increased. Apart from the increased contents of total protein and sarcoplasmic protein of *longissimus dorsi* muscles, no effect of live weight on meat quality of Large White pigs (slaughter weights of 94 and 114 kg) was noted by Choi et al. (2013), which is similar to the observations made by Correa et al. (2006) for Duroc x (Landrace x Large White) pigs of a slightly higher weight level. Comparing Duroc x (Landrace x Large White) barrows at 100 and 130 kg body weight, respectively, Čandek-Potokar et al. (1998) reported reduced a\*, b\* and C\* values of the *longissimus dorsi* with increased slaughter weight. Covering a wide range of body weights from 20 to 125 kg, Wiseman et al. (2007) found no effect of live weight on loin color except for increased b\* values. In contrast, García-Macías et al. (1996), Fischer et al. (2006) and Latorre et al. (2004) found a\* values increased with slaughter weight, supported by increasing pigment contents of the *longissimus* muscle. Slaughter weights in these studies exceeded 90 kg. Summarizing the results for Ban pigs slaughtered at live weights between 6.5 to 29.3 kg and both indicator muscles, no major changes in meat quality were detected with increasing weight. Changes in early pH fall due to altered slaughter weights were rarely observed in the available literature, but the specific slaughter conditions, such as the intense handling of the Ban pigs during slaughter and refraining from stunning them, could be partly responsible for this effect. Live weight-dependent changes in water binding capacity could possibly be related to pH1, especially alterations of CL, but a causal relationship between an early pH drop and CL in Ban pigs cannot be postulated on the basis of the available data. The absence of a significant effect of live weight on IMF corresponded to the result obtained for the development of the subcutaneous and intermuscular fat depots, and was mainly attributed to the low slaughter weights applied in this study.

#### **5.4.2 General discussion and conclusions**

Economic analyses of traditional pig production systems in the Southeast Asian Massif have shown that low cash revenues in local markets along with high opportunity costs for labor have curtailed the profits of smallholders (Lemke and Valle Zárate, 2008; Riedel et al., 2014). On the other hand, it has also been revealed that these producers would particularly benefit from rising farm-gate prices because of the low opportunity costs of the feedstuffs applied (Lemke and Valle Zárate, 2008). Increasing the quantity of the product sold and/or accessing high-priced markets could therefore provide opportunities for smallholders to generate attractive revenues from the production of indigenous pigs. Where rural markets are saturated, urban niche markets could provide a new marketing option (Neo and Chen, 2009; Phuong et al., 2014; Le et al., 2016). However, accessing urban niche pork markets requires complex logistics customized for perishable products and a continual supply of commodities. Matching supply and demand has also been reported as a major challenge for niche pork suppliers in the United States (Hueth et al., 2007). In Vietnam, pig producer associations were formed to secure supply and improve the access of smallholders to inputs, services and markets. This approach was repeatedly identified as a promising development strategy by diverse international and local organizations (Binh et al., 2007; Valle Zárate and Markemann, 2010;

Le et al., 2016; Schoell et al., 2016). Indeed, by interviewing cooperative members as well as non-members, Schoell et al. (2016) showed that approaches that incorporate the collective action of smallholders could be considered as fairly successful as evidenced by a largely positive influence on the incomes of smallholders. However, members of some cooperative groups explained that access to stable and profitable markets still remained a constraint that prevented exploitation of the full potential of collective action (Schoell et al., 2016).

Because the readiness of customers to pay high prices was coupled with certain quality requirements, product quality improvement has been identified as a prerequisite to securing the sales of indigenous pork to urban niche markets (Phuong et al., 2014; Le et al., 2016). Introducing a marketing grid or quality standard (i.e. common codes of conduct) could enable joint quality management and improve contractual security within the value chain (Springer Heinze, 2007); this would thereby provide a second pivotal component of chain upgrading besides the formation of producer associations. For low weight Ban pigs, the benchmark data compiled in this study presents a definition of product specifications that could easily be transformed into a quality standard. In the case of smallholder pig producers in Son La province, Valle Zárate and Markemann (2010) developed a concept that combined the set-up of a farmers' cooperative group with a quality control system and branding for traditionally produced Ban pork. In order to practically implement the quality control, the hogs provided by the cooperative group members would have had to have been centrally collected, slaughtered and processed in a slaughterhouse. A fairly similar approach was implemented in Cao Bang province for smallholder cattle keepers producing indigenous Hmong beef (Anh et al., 2010). The latter project, funded by the International Fund for Agricultural Development, aimed to link poor rural households with supermarkets and other quality chains by collective action and by regulating product quality using standardized production protocols (Anh et al., 2010). Both projects faced difficulties. Regarding the case of the pig producers' association in Son La, the concept of a central slaughterhouse was abandoned. When confronted with the costs for the construction and maintenance of the slaughterhouse and the uncertain forecast of demand, a private business partner withdrew from the contract with the producer cooperative group. A decline in the supply of slaughter cattle also seems to have seriously threatened the sustainability of the value chain for Hmong beef in Cao Bang. Furthermore, negotiations on a quality premium with retailers proved to be challenging (Anh et al., 2010). It has been suggested that high expectations regarding the marketability of niche meat products and underestimating the transaction costs for the logistics, slaughtering and processing, and quality control could easily result in market failure, although post-project evaluations are seldom available.

The experiences in developing the value chain for Ban pork outlined above indicate that, in order to establish products from autochthonous pig breeds in niche markets, not only the profits of producers, but also those of processors and retailers launching the product, have to be satisfactory. High transaction costs in particular threaten the profits received by downstream actors. In the specific case of the pig producers' association based in Son La, the investment needs (and risks) were drastically downscaled by contracting small-scale abattoirs for the slaughtering and processing of the pigs and using regular bus companies for the transport of chilled carcass halves from Son La province to Hanoi (Le et al., 2016). This potentially leads to the additional outcome of increasing the incomes of other small entrepreneurs along the value chain, particularly those of the slaughterers. Under the current marketing system, traders, processors and retailers pay pig producers per kg of live weight; therefore an acceptable yield of the final commodity (amount of dressed carcass or lean meat) is

crucial for compensating the incurring transaction costs. With respect to the downscaled approach, Le et al. (2016) calculated that the profit margins were sufficiently high after the costs of delivering specialty Ban pork from Son La to Hanoi had been met, but they were not exorbitant. Thus, marketing of Ban specialty pork requires further optimization. Although farmers fetch high per kg prices by selling pigs at low live weights, low revenues of 1 million VND per pig (~40 €) weighing less than 12 kg had to be accepted. It has also been shown that commodity prices per kg dressed carcass, or per kg lean meat, for traders, processors and retailers have been extremely high for the lightest pigs, thereby demanding value adding activities, for instance, building linkages with gourmet gastronomy. Taking a degressive pricing system into account, slaughter weights higher than 15 kg enable downstream stakeholders to yield increased profit margins through reduced commodity prices. To transfer benefits to producers as well, slaughter weights should be increased to slightly above 20 kg. These economic considerations highlight the benefits of introducing grids and/or standards that enhance market transparency. In the specific case of the Ban pig, the implementation of product standards would thus allow value chain stakeholders to take advantage of optimal slaughter weights and maximum economic yield. Through formal contracts, business linkages between the cooperative group and buyers could be strengthened (Springer-Heinze, 2007). However, as much as quality control systems are readily applicable in regularly equipped slaughterhouses, the question of whether small-scale slaughterers could also execute adequate product quality control remains open. In fact, informative carcass measurements and straightforward and cheap recording methods that match the capacity of small-scale slaughter operations, for instance, the two-point measurement for the determination of lean meat content (Muth et al., 2015), are available, and require only short-term training before they can be implemented. In this way, small-scale abattoirs could contribute to greater market transparency and could ensure contractual obligations are complied with within the value chain. Another crucial aspect is the safety of the pork for human consumption, i.e. the microbial quality of the pork. While comprehensive food safety standards are hardly implementable on a small-scale, we observed that certain hygiene measures, e.g., the immediate removal of blisters and blood during slaughter and the avoidance of contact between the carcass and the floor, were practiced by all three slaughterers. The feasibility of upgrading these processes in order to obtain safer products deserves more attention. The empirical data on the physico-chemical meat quality indicated that small-scale slaughterers should also be trained in handling practices to reduce the stress of the pigs before slaughter, and to avoid the occurrence of meat defects induced by the pH of the meat declining too rapidly.

Emerging, largely informal value chains for traditional pork products in northwestern Vietnam are hardly coordinated to date. This study concluded that this could result in the application of suboptimal market weights of pigs and, consequently, in reduced profits of rural smallholder producers, as well as of downstream stakeholders of the value chain. Thus, information gaps and non-transparency could threaten the sustainability of these value chains and hinder collective action strategies for pro-poor rural development to unfold their full potential. As discussed, promoting small-scale slaughterers could play an important role in closing the information gaps within niche pork value chains and improve the integration of producers and downstream stakeholders. Controlling and communicating product quality according to contractually secured standards would be a new task for slaughterers. The benchmark data required for developing such a standard for low weight pigs was documented in the empirical part of this study. The partial formalization of a value chain for niche pork, enabled through small-scale slaughterers and processors, could provide a more flexible and promising development pathway for upgrading value chains compared to 'full package'

solutions, which are often inflexible and demand (too) high investment and transaction costs. It should be noted, however, that the present study provides limited evidence to verify this hypothesis.

The coordination of breeding, management and marketing activities of native Southeast Asian pig breeds could thus be further optimized by balancing quality requirements with regard to the carcass and meat against maximum product quantity, as exemplarily demonstrated here for the Vietnamese Ban breed. Therefore, we recommend developing marketing grids and/or product standards for specific pig ecotypes at their optimal slaughter weight on the basis of practically relevant slaughter trials in other regions of the Southeast Asian Massif where similar conditions exist. These trials should take the feeding practices into account and would possibly lead to seasonally adjusted marketing weights, according to variations in diet composition. Although not elaborated on in-depth in this research, it was indicated that gradually formalized value chains for traditional pork products from low weight indigenous pigs could provide the potential not only to contribute to pro-poor rural development in the Southeast Asian Massif, but also to the conservation of animal genetic resources of a highly valuable eco-cultural region. Finally, tailor-made solutions for value chain upgrading based on solid technical-biological and economic data should be developed in order to achieve optimal value chain cooperation among stakeholders.

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## 6 General discussion

In this thesis, the potential to (re)valorize specific pig and chicken genetic resources through the production of high quality meat was explored for both species by using two approaches within different settings. The main focus was placed on the determination of genotypic differences in meat color and quality between conventional and low yielding genotypes (papers I and III). The entry point for the discussion in chapter 6.1 is therefore a reflection of the methodological approaches applied within the two studies.

In chapter 6.2, the findings of paper I - comparing two slow-growing chicken lines with a commercial broiler - are discussed, emphasizing ultimate breast meat pH and cooking loss, two traits which are of rising concern in the breeding and processing sector of meat-type chickens. These traits could relate to the selection on growth rate, particularly to weight gain of the breast muscle.

According to several scientific reports, selection on enhanced growth performance could also correlate with a reduced concentration of heme pigments in the meat of intensively selected lines and changes in their meat color. Therefore, the usefulness of color in discriminating fresh meat according to its genotype is discussed in chapter 6.3, referring to the results of the papers I and II on chickens and to those of paper III on pigs.

In chapter 6.4, the opportunities and constraints of linking smallholder pig producers of a lagging rural region in northwestern Vietnam to remunerative high-end pork markets, which provided the context for the research in papers III and IV, are outlined. In chapter 6.4.1, the results of paper III, on the discriminability of meat quality of the indigenous Ban breed, are discussed with reference to the sylvo-pastoral pig production system of southwestern Europe. For this extensive system and its local pig breeds, scientific data is abundant, enabling a contrast with the Ban production system, and the possibility to highlight the peculiarities of the latter, particularly with respect to lipid characteristics. In chapter 6.4.2, the discussion on establishing a niche market for high quality Ban pork is expanded using technical and economic aspects related to the optimum marketing weight, thereby mainly referring to paper IV.

In chapter 6.5, the general conclusions from this thesis with respect to the distinctiveness of meat color and quality from local pig and chicken genetic resources in relation to the application of specific production methods and marketing strategies are drawn.

## **6.1 Coping with confounding factors in the determination of genotypic differences in meat color and quality**

Meat quality traits are sensitive to an array of influential factors (for a review on factors influencing the eating quality of pork, see, for instance, Ngapo and Gariépy, 2008). In the statistical analysis of variance between genotypes or other experimental treatments, the correlation of one or more of these influential factors with both the dependent and independent variable results in confounding. Therefore, various pre-, peri- and post-slaughter conditions had to be taken into account in the design of both studies and in the interpretation of the results. The decision regarding whether to avoid or accept confounding variables in the respective study depended on the feasibility to do so and the specific study objectives. Consequently, two markedly different approaches for both studies were developed. The background for this is discussed in the following section.

Firstly, the choice of slaughter age is an outstanding factor in meat research, where fast-growing lines reach market weight at a younger age compared to slow-growing lines. Thus, if slaughtered at a given market weight, the genotypes will differ in their age, which is a common situation in meat research. In consequence, age represents a confounding factor that can be crucial in the expression of meat color and quality (see also chapter 6.3). In order to meet specific market requirements, not only the age at slaughter, but also the body weight at marketing often varies in practice between slow- and fast-growing lines. For instance, for the production of dry-cured pork products, both the slaughter age and market weight of traditional southern European pig breeds are increased markedly beyond those applied in the conventional sector (Bonneau and Lebret, 2010). In the comparison of purebred Ban pigs and commercial crossbreds, sired by Piétrain x Duroc boars and presented in paper III, a similar slaughter age of 6 to 7 months was chosen for both genotypes, primarily because Ban hogs intended for the high-end segment are marketed at that age. In addition, the chosen age was only slightly below the marketing age for commercial crossbreds. Due to the differences in growth rate, the slaughter weight considerably diverged between both groups and represented a confounding factor. Generally, the effect of live weight on meat quality characteristics is hardly separable from age and was accepted. In the trial comparing different chicken lines, the challenge of coping the confounding of genotype with slaughter age/weight was approached by targeting different slaughter ages according to the genotypic growth rate. Thereby, lines were slaughtered at an equal chronological age, and, additionally, at a similar body weight at slaughter by comparing younger fast-growing chickens to older slow-growing chickens. But, still, the confounding of slaughter date with age could not be avoided in this study due to logistic restrictions. It has been pointed out that a sound comparison of different genotypes would have to be carried out at the same physiological age, that is, at the same body weight relative to the weight at maturity of each genotype. This is, however, difficult to realize, because the growth potential of the investigated genotypes is frequently unknown. An attempt to estimate the growth potential has been made for the chicken lines in paper I by the application of the Laird form of the Gompertz equation (Koncagul and Cadirci, 2009) and the Morgan equation (Darmani Kuhl et al., 2010), but the latter revealed problems of convergence for data of birds slaughtered at an age of 7 weeks. Therefore, in paper I, only the parameters obtained from the sigmoidal Gompertz-Laird form are presented, despite the fact that there are also some limitations associated with its application. One constraint is represented by the fact that the point of inflexion of the curve is fixed, namely at  $1/e$  of the asymptotic weight. Additionally, the estimation of parameters, particularly of the asymptotic body weight (i.e. the growth potential), is biased in case the data is truncated before the mature body

weight is reached (Aggrey, 2002). Indeed, the estimation of growth curve parameters for the chicken lines in paper I varied according to the slaughter age applied.

Secondly, the raising of traditional genotypes is frequently associated with specific feeding practices and/or other peculiarities of the production system. For instance, Iberian pigs that are raised according to the specifications for “de bellota” ham must not receive supplementation other than natural pasture and acorns (Ministry of the Presidency Spain, 2014). In order to assess the meat and fat quality of Iberian pigs compared to those of a conventional genotype, Estévez et al. (2006) allowed for confounding through the production system, slaughter age and weight in their study. The results enabled them to make detailed recommendations on the use of raw materials for processing obtained from Iberian pigs, while a comparison under standardized conditions would have been of limited relevance in this respect. The effects of the interaction between traditional southern European pig breeds and the extensive production system on the lipid characteristics of the products are particularly considerable (Pugliese and Sirtori, 2012). Besides feeding, the temporary or permanent access to an outdoor area is also often linked to slow-growing genotypes. For instance, for poultry reared outdoors, according to the “Traditional free range” or “Free range – total freedom” regulation of the European Union (Commission Regulation (EC) No 543/2008), the use of a strain recognized as being slow-growing is prescribed. In this case, genotype effects on meat color and quality are confounded with the effect exerted by the rearing environment. Kwasiborski et al. (2008) detected an interaction between the sire breed of pigs and the rearing method (indoors/outdoors). In order to draw conclusions with a high and immediate relevancy for the situation of smallholders in northwestern Vietnam, the approach chosen for research in paper III followed that outlined by Estévez et al. (2006). This means that not only did slaughter weights markedly diverge, but the production environment provided by farmers also differed substantially between purebred Ban pigs and commercial crossbreds. Being aware of the potential impact of feeding and housing factors on meat color and quality, data on the production system characteristics associated with each of the two pig genotypes was collected at purchase. In fact, systematic discrepancies in housing and feeding between commercial crossbred pigs and purebred Ban hogs became obvious (paper III), reflecting the different production objectives of farmers. Smallholders raising Ban pigs kept costs low by the utilization of cheap and bulky feed components, and by allowing pigs to roam freely during the daytime. In contrast, the diets fed to commercial crossbreds differed considerably in the type and diversity of components when compared to the diets offered to traditional Ban pigs. Additionally, all crossbred fatteners were penned, restricting their ability to exercise. In contrast to the observational-explanatory study undertaken in Vietnam, the environmental conditions, including feeding, were standardized in the controlled trial on chicken lines in order to isolate the effect of the genotype (papers I and II). But, still, factors beyond experimental control could have exerted different effects on genotypes. In this regard, the potential confounding factor of high ambient temperatures due to the higher susceptibility of broilers to heat stress has been discussed paper I, chapter 2.4.

Thirdly, pre- and peri-slaughter effects, including events in the hours to minutes before slaughter (transportation, lairage) and those directly after slaughter (handling, stunning), as well as exsanguination, can impact meat color and quality. Thereby, pre- and peri-slaughter conditions

impose stress,<sup>12</sup> eliciting behavioral and physiological responses in the animals. The effects of these responses on muscle metabolism depend on genotype, for instance, as Terlouw (2005) reported, Large White pigs exhibited a higher stress sensitivity compared with Duroc fatteners, resulting in an elevated glycogen consumption of muscles and a limited postmortem pH decline. Similarly, Debut et al. (2005) observed an effect of genotype on behavioral response during exposure to pre-slaughter stressors. Thereby, at similar live weights, slow-growing chickens exhibited a more intense wing flapping behavior on the shackle line than fast-growing chickens (Debut et al., 2005), which increased breast meat redness and reduced curing-cooking yield (Berri et al., 2005). In contrast, under conditions of minimized stress, slow-growing and fast-growing birds did not differ in meat color and quality, and both revealed a better water retention compared to broilers from a heavy line (Berri et al., 2005). Thus, in order to elicit the direct genotypic effects on meat color and quality, the behavioral response toward stressors should be reduced. In the on-station experiment on chickens (papers I and II), pre-slaughter stress was minimized, and the birds were individually stunned. Also, in the experiments on pigs, a considerate procedure was applied, but it was more difficult to provide standardized conditions for both genetic groups. To counteract this situation, the number of animals slaughtered per day was limited and, on most slaughter days, mixed groups were slaughtered. Since the small-scale abattoirs exhibited slightly different facilities and slaughtering practices, slaughter was conducted in three different places in total. Again, the different body size of commercial crossbred compared and Ban hogs represented a confounding factor that could have interacted with the slaughter conditions, and, indeed, as shown in paper IV, live weight had a significant effect on the early pH development of Ban pigs under the slaughter conditions in place.

The confounding factors may have attenuated or amplified genotypic differences in meat color and quality traits; therefore, linking results from field data to single factors, such as the genotype, is aggravated. Being aware of the trade-off between the establishment of causal relationships and the relevancy of results for practical situations, which is considerably shifted in favor of the latter for explanatory-observational studies (Dohoo et al., 2003, p. 142), it was decided to accept these restrictions in the pig study design. Because the study was embedded in a transfer project, and due to its connectedness with a marketing study (Huyen et al., forthcoming), priority was placed on the collection of data with practical relevancy. In contrast, inference on isolated factors should have been enabled in the chicken experiments; therefore, a controlled trial was designed that attempted to reduce confounding variables as much as possible. This was not completely achieved, but the caveats have been pointed out.

## **6.2 Are the differences in functional breast meat quality between slow-growing strains and a broiler related to growth performance?**

In this chapter, differences among slow- and fast-growing chicken lines presented in paper I, chapter 2.3, with a focus on ultimate pH and cooking loss are discussed, and broadened by the question of how far these traits could relate to the growth performance of chickens. Within this context, the potential to discriminate the products from local chicken breeds by these traits has to be considered. The slow-growing genotypes were represented by two experimental chicken lines maintained by the Institute of Animal Production in the Tropics of Subtropics, University of Hohenheim, whereas the fast-growing genotype was represented by a commercial broiler (for detailed information see paper I,

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<sup>12</sup> Stress can be defined as “[...] the physiological, behavioral and psychological state of the animal when confronted with a potentially threatening situation” Terlouw (2005, p. 126).

chapter 2.2). All chickens were sexed at birth and, in total, 264 male birds were utilized for the experiment.

Because of its relationship with the color and water holding properties of chicken meat (Fletcher, 1999; Wilkins et al., 2000; van Laack et al., 2000; Qiao et al., 2001; Berri et al., 2005; Berri et al., 2007; Janisch et al., 2011), ultimate pH is considered as a central quality trait for poultry meat with a narrow window of optimum values: too high or too low values indicates DFD-like or PSE-like conditions,<sup>13</sup> respectively. Therefore, the pH assessed at 24 h postmortem has been repeatedly suggested for application as a selection criterion within breeding programs for chickens (Le Bihan-Duval et al., 2013; Phongpa-ngan et al., 2014). The results of paper I show that the pH value of chicken breast meat at 24 h postmortem was substantially influenced by both line and slaughter age/weight. The latter effect was particularly evident in the fast-growing broiler, whereas the final pH value of breast (paper I) and thigh meat (Muth and Valle Zárate, 2014) increased with slaughter age/weight. Similar results for broiler breast meat have been obtained by Baéza et al. (2012), but not by Schneider et al. (2012). At the same chronological age, the pH of fast-growing chickens was significantly higher compared to both slow-growing lines, but the differences were negated or even reversed when similar body weights at slaughter were considered (paper I). Firstly, these results support the hypothesis that myofiber hypertrophy could be linked to the expression of ultimate pH through the reduction of the glycolytic potential as suggested by Berri et al. (2007), Le Bihan-Duval et al. (2008) and Baéza et al. (2012). Secondly, these results suggest that the variation of slaughter age/weight relative to the inflexion point of the growth curve could at least partly account for the contradictions among scientific literature with respect to the genotype effect on the ultimate pH of chicken breast meat. It could be speculated that the differences between slow- and fast-growing birds are more likely to be expressed when broilers, which showed an extreme maximum daily gain, had passed this point. Thirdly, it is indicated that for genotypes utilized to produce different target weights, for instance, for the production of whole carcasses or cut-up parts, the age/weight-dependent variation in pH could result in diverging meat quality. Given the small numerical difference in the average ultimate pH between the bird groups (i.e. line and age combinations) under investigation, and the limited variation in this trait, it is questionable whether this bears practical relevance, for instance, for application in breeding programs. The applicability of colorimetric measurements as proxy for ultimate breast meat pH was investigated further on a subsample of the data in paper II, whereas rather weak residual correlations of colorimetric values with pH were revealed. Furthermore, the phenotypic association of L\* values with pH depended on the interactions between the measuring position and genotype as well as storage. The results indicated that, before the practical application of colorimetric measurements to predict ultimate pH in chicken breast meat, a calibration with respect to the measuring position on the fillet has to be carried out separately for each genotype.

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<sup>13</sup> Smith and Northcutt (2009) pointed out that “PSE” (pale, soft, exudative) is terminology that has been developed specifically for a meat defect of pork, and, due to major differences in the underlying causative mechanism(s) and in the extent to which the defect is expressed, the term PSE should not be applied to chicken meat. They suggested utilizing either the term “pale chicken muscle” or “pale poultry muscle syndrome” to describe pale meat that exhibits a low pH and a reduced water holding capacity (Smith and Northcutt, 2009). This view is widely accepted nowadays; however, in practice, the terms “PSE-like” or “DFD-like” for the dark, firm, dry defect have been adopted to describe these defects in poultry meat.



The association between pH and growth performance was investigated further by separately calculating residual correlations of breast meat pH with the Gompertz-Laird growth curve parameters for each line (paper I). The results supported the hypothesis of an association between growth performance and breast meat pH. For all lines, a suspended point of inflexion and an increased growth potential was related to an increased ultimate pH value. Recently published results of an experiment on two female broiler lines from the same base population, which were selected for high and low breast meat pH (Alnahhas et al., 2014), appear, at least in parts, to contradict this statement. Alnahhas et al. (2014) showed that after five generations of divergent selection for pH, body weight at 6 weeks of age was not different between both of the lines. Yet, the breast meat yield of the high-pH line was significantly increased compared to the low-pH line (Alnahhas et al., 2014). Moreover, while Phongpa-ngan et al. (2014) found no correlation between ultimate pH and body weight at an age of 6 weeks for a slow-growing subpopulation of the experimental “Arkansas randombred” line, the ultimate pH was positively correlated with body and breast weight, as well as with breast yield (0.17, 0.25 and 0.19, respectively) for the fast-growing subpopulation of that study. Indeed, in paper I, a high maximum daily growth rate correlated positively to ultimate pH of the broiler, whereas for both slow-growing lines this correlation was close to zero. The maximum daily growth rate is probably linked to the disproportionate growth of the pectoral muscle and, thus, to breast meat yield, which could connect the results of both aforementioned studies (Alnahhas et al., 2014; Phongpa-ngan et al., 2014) with paper I. In summary, it appears that the increased pH observed for the broilers compared to the slow-growing birds at an equal chronological age (paper I) is related to the accelerated breast development of fast-growing chickens due to radial myofiber growth, and particularly expressed at high body and breast muscle weights. It seems to be unlikely that selection on increased growth performance of slow-growing chicken breeds would reduce pH. Given the limited variability of ultimate pH values, the inclusion in a breeding program seems to be, as suggested by Harford et al. (2014), rather reasonable on the basis of families compared to its application on the individual level.

Another interesting aspect resulting from the comparative analysis of chicken lines were the increased cooking losses noted for breast meat (paper I) and for thigh meat (Muth and Valle Zárate, 2014) of broilers compared to both slow-growing strains. Unlike the results for pH and other functional meat quality traits, these differences were expressed irrespective of the slaughter age/weight. Although a relationship between impaired water holding capacity and decreased ultimate pH values is well established in scientific literature (van Laack et al., 2000; Woelfel et al., 2002), increased cooking losses were expressed concurrently to high ultimate pH values of breast meat of broilers aged 49 to 70 days (paper I). This configuration of broiler breast meat functionality resembles the “white striping” condition of heavy broilers, a defect which was first described by Bauermeister et al. (2009) and recently investigated by several authors. This meat defect and the extent of its severity have not only been linked to the characteristic white striation on the surface of breast fillets (Petracci and Cavani, 2012), but also to increased cooking losses (Mudalal et al., 2014; Mazzoni et al., 2015; Mudalal et al., 2015) and increased pH (Mudalal et al., 2015). For the wooden breast defect, which sporadically co-occurs with white striping, suggesting a similar histopathologic manifestation, Sihvo et al. (2014) observed extensive polyphasic myodegeneration with phases of regeneration, as well as inflammatory processes. All of the broilers from four of the most popular commercial strains exhibited at least mild signs of myodegeneration in the primary myofiber fascicles of the breast muscle (Mazzoni et al., 2015). Thus, emerging myopathies could impose issues for the broiler industry, with severe cases being affected by the wooden breast or white striping defect, and

milder cases displaying elevated cooking losses. Besides the reduction in cooking losses, no other adverse effects on technological quality have been observed for the white striping condition. Mudalal et al. (2015) even reported decreased drip loss, which could be related to increased ultimate pH values. With respect to the chemical composition, an increase in lipid content for the more severe forms and a reduction in protein concentration are notable (Mudalal et al., 2014; Mazzoni et al., 2015). Interestingly, Kuttappan et al. (2012) reported that the frequency of severe cases of white striping was increased by a feed-induced acceleration of the growth rate. In addition, strain differences in the occurrence of severe cases of white striping (Kuttappan et al., 2013), as well as the increased breast fillet weights observed for birds affected by white striping (Mudalal et al., 2015), point toward an involvement of the growth rate in the etiology of the white striping defect. The occurrence of white striping was not recorded in the present study, but it can be speculated that the elevated cooking loss of broilers compared to the slow-growing experimental lines (paper I) could be a “subclinical” form of white striping and be related to the increased growth rate of broilers. The recent emergence of the white striping defect within the broiler sector further supports the suggested relationship between growth rate and meat quality deteriorations of this novel issue, assuming that improving growth efficiency continues to be an important goal in broiler breeding. In contrast to the results of paper I, there are also numerous studies which attributed reduced cooking losses to fast-growing lines instead of slow-growing strains. For instance, the fast-growing subpopulation described by Phongpa-ngan et al. (2014) had a better cooking yield than the slow-growing subpopulation. It could be suggested that growth rate and cooking loss follow a complex non-linear association. Indeed, also found in paper I, a negative (i.e. favorable) association between cooking loss and maximum daily growth rate was detected for both slow-growing lines, while this relationship was not significant for the fast-growing line. The populations investigated by Phongpa-ngan et al. (2014) had overall growth rates that were intermediate between the slow- and fast-growing lines of the present study. This could offer an explanation for the contradictions in the results for cooking loss between Phongpa-ngan et al. (2014) and paper I, and further supports the hypothesis of a non-linear relationship between cooking loss and growth rate. On the other hand, cooking loss could also be affected by other genetic factors than those determining growth traits. Some possible additional explanations for factors that could influence cooking loss are subsumed in paper I, chapter 2.4. In summary, the issue of increased cooking loss – even if not accompanied by severe myodeneration – can be relevant for the broiler industry. Through scale effects, the economic impact due to weight loss during processing can be considerable. The consequences on eating quality remain to be elucidated. Thus, possibly, the reduced cooking loss could represent an attribute that allows for the discrimination of meat from slow-growing lines deriving out of commodity produce at present, depending on the ability of the broiler sector to respond to this situation.

Despite the strong indication that the water holding capacity of breast and thigh meat from fast-growing broilers during processing is compromised as a result of thermal impact compared to the slow-growing chickens, the conclusion that selection on high growth performance would deteriorate meat quality *per se* could not be drawn. Firstly, cooking loss was the only trait that was adversely expressed by the broiler irrespective of the slaughter age/weight. In regard to dependence on slaughter age/weight, other traits were even favorably expressed for the fast-growing line, for instance, at an age of 10 weeks, the cooked breast meat of fast-growing broilers was tenderer when compared to the meat of one of the slow-growing strains (paper I). There were also variations noted for breast meat functionality among the slow growing lines, despite their similar growth rates and breast meat yields. Secondly, the strength of the residual relationships between growth curve

parameters and functional meat quality traits were, if a statistical trend was detected at all, rather low, with the correlation coefficients of residuals ranging from 0.17 to 0.33 (paper I), indicating that meat quality is, in part, determined independently from growth. Furthermore, the association of meat quality traits with growth curve parameters appears to be genotype-specific in most cases. Thirdly, within lines, an increased growth potential was frequently positively related to meat quality aspects, particularly for the broiler, for which an increased growth potential was favorably correlated to cooking loss, expressible moisture and tenderness (paper I). Representing a hypothesis that must still be verified, the rationality and feasibility of an attempt to simultaneously improve growth performance and meat quality via selection on increased growth potential has to be questioned. As indicated in chapter 1.2, it has to be considered that improving growth potential is not necessarily of economic relevance as meat-type chickens are slaughtered before reaching their adult body weight. In view of the rather low correlations, corresponding to the only sporadically noted genetic correlations between growth rate and meat quality attributes, meat quality could, probably, be genetically improved independent from growth performance, especially with respect to slow-growing chicken lines.

### **6.3 How useful is the color of fresh meat for discriminating specific pig and chicken genetic resources from conventional genotypes?**

Besides shape and surface characteristics, color is a main aspect that determines a food item's quality from a customer's point of view (Lozano, 2006). Therefore, the improvement of meat color and its maintenance during display are of high economic relevance. This is particularly relevant for niche products because of the ability to distinguish them from commodity produce by their color on the retail shelves, and the interplay of meat color with extrinsic quality cues that allow customers to differentiate products at purchase (see also chapter 1.1). The objective measurement of meat color in research and the industry is typically conducted by Minolta (papers I, II and III) or Hunter colorimeters, and meat color is reported according to the Cartesian CIE (Commission International D'Éclairage)  $L^* a^* b^*$  color space ( $L^*$  = lightness,  $a^*$  = redness,  $b^*$  = yellowness) (Tapp III et al., 2011). Details on the instrumental assessment of meat color are referred to in paper II, chapter 3.1. Thus, where variation among genotypes exists, meat color could be utilized to discriminate niche products derived from slow-growing breeds/genotypes from those of conventional genotypes, and instrumental color values could represent important traits when deciding which to incorporate within breeding programs and marketing strategies. Against this background, the hypothesis that selection on growth performance correlates to a reduced concentration in heme pigments in the muscles of monogastric species (Oksbjerg et al., 2000; Berri et al., 2001) is noteworthy, primarily for two reasons: firstly, alterations of the heme pigment level and/or pH values could be associated with changes in color and color stability of the meat (see Berri et al., 2001 for chickens and Lindahl et al., 2001 for pigs). Secondly, a decline in heme-bound iron content could decrease the nutritional value of meat because appropriate dietary levels of iron are essential, and due to the higher bioavailability of heme-iron relative iron complexes from plant-based diets (Biesalski and Nohr, 2009). The alteration of both the aesthetic attributes of meat and of its nutritional value could be relevant for the positioning and marketing of niche products from pig and chicken genetic resources.

By analyzing the association of (Minolta) color values of chicken breast meat with several selected physico-chemical meat quality traits simultaneously for a subsample of the data set of paper I by Partial Least Squares Regression (Wold et al., 2001), an inverse relationship of the ultimate pH and hemoprotein concentration with  $L^*$  and  $a^*$  values was detected (Muth and Valle Zárate, 2013). This

reciprocal effect of pH and hemoprotein content on meat color seems to be similar for pig and chicken meat. But, how can this effect be explained? According to Swatland (2004), reflectance by myofibrils and refraction at the phase transitions from membranes to sarcoplasm and from sarcoplasm to myofibrils is a major cause of light scattering of meat. Thereby, light reflectance and scattering were significantly affected by pH in porcine myofibers (Swatland, 2003) and chicken breast meat (Swatland, 2008). Swatland (2003) suggested that a pH-induced shrinkage of the myofibril lattice due to decreasing negative charge repulsion increases the refraction of light. When, in contrast, the light path through meat is not reduced due to incomplete postmortem glycolysis, the altered spacing of the myofibril lattice allows for an increased transmission of light and absorbance by sarcoplasmic proteins, such as myoglobin (Swatland, 2004). The variation in lightness of minced pork was primarily explained by the variation in the absorption of light at a wavelength of 525 nm (Karamucki et al., 2013). Due to the fact that at a wavelength of 525 nm the spectral curves of the redox forms of myoglobin intersect, the absorbance value is directly proportional to the pigment concentration of meat (Krzywicki, 1979). In order to better explain the development of meat redness and yellowness, the role of heme pigments and its redox forms is particularly crucial. The contribution of hemoglobin to the color of fresh meat is often minor when compared to that of myoglobin, but this varies according to the species and muscle under consideration. Hemoglobin and myoglobin reveal some similarities in their chemical properties, which aggravate their separation (Beutling et al., 2000). As Lindahl et al. (2001) indicated for porcine muscle, not only is the concentration of meat pigment of importance, but also the prevailing redox form of hemoproteins. The heme group contains a central iron ion bound to a protoporphyrin ring at four coordination sites and to the apoprotein by a histidine residue at the fifth coordination site (Faustman and Cassens, 1990). At the sixth coordination site, the heme iron is capable of binding a ligand, which determines the prevailing redox form of myoglobin. Deoxymyoglobin is the predominant form in freshly cut meat when no ligand is present and the iron ion is in a ferrous state, while oxygenation to oxymyoglobin occurs when oxygen binds at the unoccupied site of the iron ion, resulting in a cherry-red color. Through oxidation at low oxygen partial pressures, both previously mentioned forms convert into metmyoglobin, resulting in discoloration. The ligand is then represented by a water molecule and the iron ion is in its ferric state and physiologically inactive (Faustman and Cassens, 1990). In turn, metmyoglobin can be reduced to the active form by enzyme complexes (metmyoglobin reducing capacity, reviewed by Bekhit and Faustman, 2005). Furthermore, the pH value of meat is associated with the redox dynamics of hemoproteins, for instance, as Gutzke and Trout (2002) demonstrated, autoxidation of myoglobin was increased at reduced pH.

The differences in the ultimate pH between slow-growing light-skinned chickens and the commercial broiler were significant, but small (paper I). Given the limited differential in pH, it is not surprising that the differences in meat lightness were also low, although the residual correlation coefficients between both traits were mostly quite weak (paper II), indicating that other factors could also have exerted substantial influence on the expression of lightness in chicken breast meat. Indeed, the  $L^*$  values of slow-growing chickens were marginally lower than that of broilers, even though the pH of the broiler was significantly higher compared to the slow-growing chickens at an age of 7 and 10 weeks (paper I). Although the chicken breast muscle – particularly that of fast growing birds – is largely composed of type IIb fibers (Zhao et al., 2012), low concentrations of, on average, 10 ppm hemin, which is the oxidation product of heme in acidified acetone solutions (Cornforth, 2001), were detected in the pectoral muscle of slow- and fast-growing chicken lines (papers I and II). The concentrations of both hemin and soluble myoglobin were weakly positively correlated to the

expression of the  $a^*$  value of chicken breast meat (paper II), which is in line with the established relationship between hemoprotein content and meat redness. But, the differences in heme pigment concentration in the breast muscle of commercial broilers compared to slow-growing chickens were only significant when comparing one of the two slow-growing lines to a commercial broiler at an age of 7 weeks at slaughter (paper I). The absence of a marked difference in hemin concentration among the genotypes, despite the considerable variation in growth performance, is in contradiction to the observation of the reduced heme pigment concentration of selected lines made by Berri et al. (2001). It has, however, to be noted that Berri et al. (2001) drew on results derived from a selection experiment, while different genetic backgrounds had to be accepted in this work (see also chapter 6.1). But, the residual correlation coefficients of the heme pigment content with growth curve parameters within the genotypes were also around zero, and, likewise, did not point toward a strong association of growth performance with the pigment concentration in chickens (paper I). Consequently, the differences in the instrumental redness values of meat when comparing the light-skinned chicken lines with diverging growth rates were below one unit and mostly not significant when the genotypes were slaughtered at an equal chronological age. However, the differential could just be sufficient to visually discriminate meat with respect to its redness (Zhu and Brewer, 1999). The breast meat redness of broilers slaughtered at an age of 4 weeks was significantly increased when compared to slow-growing chickens slaughtered at 7 and 10 weeks of age. The hemin concentration was also slightly, but not significantly, augmented in the breast meat of 4 week-old commercial broilers, which could explain the slightly elevated  $a^*$  values of their meat. The concurrent results obtained for the hemin concentration and the  $a^*$  values of thigh meat are notable (Muth and Valle Zárate, 2014). Possibly, genotypic differences in susceptibility to stress under specific slaughter conditions (shackling) as demonstrated by Berri et al. (2005), resulted in increased blood circulation and contributed to the differences in meat color, particularly regarding  $a^*$  values. Alvarado et al. (2007) showed that improper bleeding led to an increased concentration of trapped hemoglobin in the breast muscle and to elevated  $a^*$  values in chicken breast meat. In the present study, stress at slaughter was minimized, and birds were individually stunned, which reduces the effects of genetic differences in the behavioral response of slaughter conditions (Berri et al., 2005). With respect to the increased  $a^*$  values of the 4 week-old commercial broilers, it is interesting to note that Werner et al. (2011) also reported increased breast meat redness of younger broilers (aged 28 days) compared to older ones (aged 41 days). They explained this variation as a difference in the oxidative status of chickens. Data on commercial broilers taken for a period of 35 until 63 days of age and published by Baéza et al. (2012) on growing broilers revealed some significant differences in the tristimulus color values, but there was no clear age-related trend found for the expression of individual color components. Overall, because the ultimate pH and the hemin concentration were not markedly affected by genotype, the differences in instrumental  $L^*$  and  $a^*$  values were equally weakly expressed when comparing light-skinned chicken lines at an equal age.

Relative to the lightness and redness of chicken breast meat, yellowness is more difficult to allocate in the color space of chicken meat (Muth and Valle Zárate, 2013) and deserves further attention in research. Interestingly, with respect to the genotype, the residual correlation between water-soluble myoglobin concentration and instrumental yellowness of chicken breast meat was reversed, i.e. positive for a fast-growing broiler and negative for a slow-growing chicken line (paper II). A refined QTL analysis carried out by Le Bihan-Duval et al. (2011) suggested the BCMO1 ( $\beta,\beta$ -carotene 15,15'-monooxygenase 1) gene and its promoter region as a potential candidate gene for the expression of meat yellowness, and, to a lesser extent, also of meat redness. Indeed, a mutation in the upstream

region of BCMO1 resulted in genotypes that differed significantly in carotenoid content and yellowness of breast meat. The differences detected between the homozygous and heterozygous genotypes suggested an intermediate mode of inheritance (Le Bihan-Duval et al., 2011). Furthermore, Jiali et al. (2012) found that the effects of the mutation were restricted to the breast muscle, although the activity of this gene was lowest in this particular tissue. The involvement of carotenoid metabolism indicates that enrichment of diets with components containing carotenoids could be effective in manipulating meat color, particularly in lines that exhibit the mutated variant of the BCMO1 gene at a higher frequency. In paper I, an age-related decrease in instrumental  $b^*$  values was noted for both the commercial broiler and the slow-growing light-skinned line. In fact, the experimental birds were fed a starter diet based on corn, which is a source of lutein/zeaxanthin. In contrast, the grower diet, fed from week 3 to 10, was cereal-based. Thus, an accumulation of carotenoids in breast meat could represent a confounding variable with respect to differences in meat yellowness between age groups. The yellowness of breast meat did not differ between the slow-growing light-skinned line and the broiler at equal chronological age, but was significantly increased for the younger birds, irrespective of the genotype (paper I).

Thus, considering the results for the light-skinned slow- and fast-growing chicken lines presented in paper I, there is limited evidence of a genotypic divergence in meat color. Slaughter of the lines at varying body weight could nevertheless exert noticeable differences. For instance, in the present study, younger broilers had a lighter and redder breast meat compared to older slow-growing chickens and revealed a markedly increased yellowness of breast meat. However, given the different composition of the starter and grower diet, an effect of feeding on the enrichment of breast meat with carotenoids has to be taken into account. The increased redness of younger broilers could also be partly related in particular to the slightly, but not significantly, increased hemin concentration. Still, the ability to discriminate such products by human vision is not granted because, as O'Sullivan et al. (2002) demonstrated for pork, the tristimulus values measured by a Minolta-branded instrument only partly relate to the sensory description of color.

One of the slow-growing experimental lines (slow-growing dark-skinned) possessed a combination of two major genes (the fibromelanosis gene and the inhibitor for dermal melanin), which resulted in the migration of melanoblasts into various body tissues and in dermal hyperpigmentation (Dorshorst et al., 2010). Thus, the skin and meat color of that line strongly diverged from both normally being light-skinned genotypes (paper I). The lightness and, to a lesser extent, also the redness of skin and breast meat were reduced, and the  $b^*$  values were negative, indicating a blue instead of a yellow color. Łukasiewicz et al. (2015) presented the color data of an Ayam Cemani x Sussex hybrid, exhibiting, according to published photographs, a similar carcass and meat color compared to that of the slow-growing dark-skinned line; but the reported color data strongly diverged from the data presented herein. The positive  $b^*$  values that Łukasiewicz et al. (2015) recorded are especially difficult to explain, but could be related to the instrument used for the measurement and/or its setting. The specific color of skin and meat of these genotypes are an important quality attribute because they allow for straightforward visual discrimination. In certain markets, for instance in East and Southeast Asia, dark-skinned carcasses fetch high price premiums. The appreciation of chicken skin and meat color by consumers is, however, strongly dependent on their cultural background (Kennedy et al., 2005).

Pork from the purebred Ban pig breed exhibited only subtle color differences compared to a commercial crossbred slaughtered at a similar age (6 to 7 months), although the difference in

productive traits between both groups was immense (paper III). The subjective color assessment between both genetic groups differed neither for the longissimus thoracis et lumborum nor for the semimembranosus muscle. In addition, the cross-section of loin did not differ in instrumental color values, while the cut surface of the semimembranosus of the traditional Ban breed exhibited only slightly elevated redness and yellowness values, resulting in an increased color saturation ( $C^*$ ) compared to the commercial crossbred. Referring to Swatland (2003), the absence of a difference in  $L^*$  values between purebred Ban pigs and commercial crossbreds could be partly explained by the largely concurrent postmortem glycolysis. In fact, a number of studies comparing modern breeds or commercial crossbreds to local pig breeds, particularly the Iberian breed, obtained more or less pronounced differences in meat color, whereas meat lightness was frequently decreased and redness was increased for the rustic genotype (see paper III, chapter 4.4). However, it has to be considered that in many studies the live weight at slaughter was standardized, i.e. pigs of the traditional breed were slaughtered at a significantly higher chronological age. Thus, age represents a confounding factor because the myoglobin level in porcine muscle could depend on the animal's age, as suggested by Mayoral et al. (1999) and Fischer et al. (2006). From their meta-analysis, Trefan et al. (2013) concluded that there is a quadratic effect of cold carcass weight on meat redness, whereas  $a^*$  values increased from about 110 kg onwards. They also found a positive linear relationship between yellowness and cold carcass weight, which approximates the findings by Wiseman et al. (2007). In paper IV, no effect of live weight on the meat color of the loin or ham was detected, but this could be due to the limited span in live weight and slaughter age. Variation in meat color of the Ban breed could nevertheless be generated by substantially increasing the slaughter age, as for hogs produced for the regional market. In the case of the Iberian breed, the strong effect on meat redness could, nevertheless, be related to its specific genetic background. Ovilo et al. (2002) demonstrated an effect of an "Iberian allele" on SSC4 on pigment concentrations and  $a^*$  values, which could contribute to the dark red color of the meat of this breed. However, this thesis does not support the presence of a specific allele that affects pigment concentration and meat color of indigenous Ban pigs. The slight differences in the instrumental  $a^*$  and  $b^*$  values and the color saturation of the semimembranosus of Ban pigs were not confirmed through a subjective color assessment (paper III). There was no difference between Ban pigs and commercial crossbreds in the concentration of soluble myoglobin in the loin, which, in contrast to the results of Oksbjerg et al. (2000), does not support the hypothesis of a major effect in the selection of growth traits on pigment concentration. Overall, the magnitude of values for soluble myoglobin in the loin was in line with that reported by Newcom et al. (2004), who found significant breed differences for this trait. There was at least a statistical tendency of a slightly augmented soluble myoglobin concentration in the semimembranosus of Ban pigs (+21%; paper III). The slightly increased pigment content and redness of the semimembranosus of the Ban pigs could indeed be explained by an increased proportion of slow-twitch oxidative myofibers (Gil et al., 2008) compared to the commercial crossbreds, or, since the semimembranosus is a muscle of the hindleg, by an increased physical activity of Ban pigs due to semi-scavenging or rearing in spacious enclosures (Thomas et al., 2013). Leuret et al. (2011) reported higher yellowness and redness values in the semimembranosus muscle of pigs reared on bedding and with access to free-range conditions compared with pigs reared indoors on a slatted floor. On the other hand, in a study by Bee et al. (2004), the outdoor rearing of pigs in a cold environment had some effects on fiber type distribution, muscle metabolism, and on the meat quality of different hindleg muscles and the loin, but color was only marginally affected. Thus, the partly semi-scavenging management of Ban pigs could have possibly resulted in a small effect on the color of the ham of the Ban breed through increased physical activity. Other husbandry-related effects on meat color, such as the sporadic or permanent

feed restriction of Ban barrows, seem to be rather unlikely. Serrano et al. (2009) did not detect an effect of feed restriction on the color of the longissimus muscle in pigs, suggesting that for meat color the effects of restriction of feed intake seem to be negligible. Thus, the differences in color and pigment concentration between both pig genotypes were quite small. Given the relatively close association of myofiber traits and meat color (Nam et al., 2009), the similarity in meat color did not indicate a pronounced divergence in the composition of muscles between both breeds. This was surprising in view of the marked variation in the genetic background and production traits of both groups.

Overall, the differences in heme pigment content among slow-growing chicken lines and commercial broilers, as well as the rustic Ban breed and crossbreds sired by modern Piétrain x Duroc, were relatively small and mostly insignificant. The results presented here, with careful consideration to avoid overinterpretation, do not speak in favor of a profound effect of selecting for growth performance on the oxidative metabolism of muscles and the heme pigment content of modern pig and chicken genotypes. The results from pigment extraction were confirmed by the low differences in instrumental meat redness. It seems that, if no major gene(s), as in the case of the dark-skinned slow-growing chicken line, exists, a more distinctive meat color can be generated instead by altering additional features of the production system, for instance, slaughter age, feeding, and/or providing access to exercise.

#### **6.4 Linking smallholder pig producers of the northwestern Vietnamese uplands to urban pork niche markets: opportunities and constraints**

The pig trials were embedded in a transfer project<sup>14</sup> funded by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG). The project was aimed at establishing a community-based pig breeding and marketing cooperative in a lagging rural region of northern Vietnam (Valle Zárate and Markemann, 2010). The mixed crop-livestock farmers who participated in the cooperative belonged to an ethnic minority, the Black Thai (Thái Đen), and could be roughly divided into two groups according to the objectives they pursued regarding pig production. Farmers who were located in the vicinity of Son La city – the provincial capital of Son La province – exhibited clear market-orientation, whereas farmers from more remote districts practiced resource-driven livestock husbandry for home consumption, the generation of savings, and to fulfil socio-cultural purposes (Lemke et al., 2006). The market-oriented group produced leaner crossbreds with a relatively high input level for the regional market, whereas the remote farmers, in contrast, kept the Ban pig breed under low-input low-output conditions (Lemke et al., 2007). The switch of farmers with access to urban markets to conventional genotypes, primarily used in unstructured crossbreeding programs (Berthouly-Salazar et al., 2012), was correspondingly reflected in a sharp decline of local breeds. In the northwestern uplands, the indigenous Ban breed was the only breed in the 1950s, but by the early 2000s the proportion of Ban keepers had already halved, and their occurrence is now restricted to resource-driven farming systems (Lemke and Valle Zárate, 2008). The main income of the remote farmers was derived from cash crop production (e.g., coffee) and paddy rice (Lemke et al., 2006).

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<sup>14</sup> Transfer projects are a project type funded by the German Research Foundation. Within Collaborative Research Centers (Sonderforschungsbereiche), transfer projects “provide a platform for researchers to test results obtained through basic research under practical conditions or to develop prototypes. Through research carried out jointly by academics and application partners, these projects aim to improve the mutual exchange between of applied research back into basic research” (German Research Foundation, 2013).



These households marketed only a small share of their pigs, mainly supplying rural outlets via a complex network of intermediaries (Huong et al., 2009; Huyen et al., forthcoming). Given their dependency on the development of world market prices for cash crops, along with limited opportunities to obtain off-farm income, the vulnerability of remote farmers' livelihoods is high (World Bank, 2012). Additionally, farmers of the northwestern mountains are prone to a number of natural disasters that impose a threat on crop production. In the period from 2003 to 2008, drought, flood/storms, landslides and other forms of extreme weather were experienced by 8.1, 14.3, 1.3 and 22.6% of households in this particular region, respectively (Vietnam Household Living Standards Survey, 2008 cited in World Bank, 2012). Thus, diversification into additional income generating activities, including specialty pork production, could be an important strategy in improving the resilience of their livelihoods (Keil et al., 2011). Niche marketing of value added animal-based products has been pointed out as a development option for smallholders in various texts (e.g., see FAO, 2011), but scientific documentation of such initiatives in developing countries is, at present, rare. In the Chinese Yunnan province – also part of the Southeast Asian Massif –, a development program to diversify the income of smallholder pig keepers by specialty pork production failed (Neo and Chen, 2009). But, within that project, the market potential for the “micro-variety” of the “Small Ear Pig” breed (Riedel et al., 2012), an ecotype similar to the Ban breed, was clearly demonstrated. It is assumed, therefore, that similar potential exists in opening up new, remunerative markets for Ban pork in the urban lowlands of northern Vietnam, particularly in Hanoi. Thus, specifically, the households of the farmers' cooperative located in the remote zones who kept the local Ban breed were addressed by combining meat research and marketing activities in order to provide access to remunerative niche markets for indigenous pork (Valle Zárate and Markemann, 2010).

Previous market research showed that affluent urban customers, as well as gourmet restaurants, in the lowlands of northern Vietnam could represent potential buyers of native specialty pork (Lapar et al., 2010; Pedregal et al., 2010; Phuong et al., 2014). Recognition of the product by producers, retailers and consumers is one of the key factors for the establishment of new nested markets (van der Ploeg et al., 2012), and, hence, appears to be met in the case of Ban pork. In contrast to traditional European pig breeds, for which market weights often exceed those applied within conventional production systems (Bonneau and Lebret, 2010), Ban pigs dedicated for the lowland specialty market are purchased at low body weights of only 10 to 16 kg (Huyen et al., forthcoming). Because of the extremely low daily weight gain of <100 g/day of purebred Ban pigs reared under extensive conditions (see paper III and Lemke and Valle Zárate, 2008), they attain a relatively high average age for marketing at approximately 7 to 10 months (Huyen et al., forthcoming). Yet, retailers in Hanoi paid more than twice per kg for lightweight Ban pigs compared to the price realized for heavier porkers in Son La (Huyen et al., forthcoming). A longitudinal study among restaurants in northern Vietnamese lowland provinces showed that the restaurant owners paid higher prices for pork from purebred Ban pigs compared to pork from crossbreds or for conventional pork (Phuong et al., 2014), creating an incentive to supply this market niche. But, *inter alia*, the lack of documentation for specific characteristics, by which the quality of native Ban pork can be defined and discriminated, represented major shortcomings. Consequently, in the case that the product realizes attractive prices, but is not distinguishable on the market by intrinsic or extrinsic quality cues, free riders can severely damage the benefits created by niche markets (van der Ploeg et al., 2012), for instance, by mixing pork from different sources (Phuong et al., 2014). Ultimately, this could result in market failure. In addition, eligibility for governmental subsidies paid for the conservation of national genetic resources, depends on scientific proof and recognition of the quality of breed-specific products and

their valuation by the market (Huyen et al., 2005). Therefore, paper III addressed the question of which traits Ban pork color and quality can be distinguished from commercial pork.

Besides positioning the product on the market, it is equivalently important to fairly distribute the return on sales among the actors of the value chain, including the primary producers, in order to establish a short supply chain for Ban pork. This requires a transparent price formation that can be, for instance, achieved through carcass classification (Eikelenboom et al., 2004). In the determination of the optimum live weight at marketing, trade-offs between product quantity and quality have to be considered. Producers aim at increasing the market weight of pigs because in this way the meat output per sow can be maximized; but, at the same time, they have to ensure market-conformity of their produce (Eikelenboom et al., 2004; Gonzàles et al., 2014). When the producers fail to deliver finishers that provide features in compliance with the market requirements, for instance, due to information asymmetries, market failure can occur. For retailers and gourmet chefs of fresh, indigenous pork in the northern Vietnamese lowlands, the lean meat percentage is an important quality attribute for which they desire a minimum of 40% (Huyen et al., forthcoming). The prioritization of relatively high lean meat percentages was the reason for the preference of light slaughter weights of Ban pigs by urban high-end markets (Phuong et al., 2014), because at relatively high body weights of 50 kg the lean meat percentage had drop to only 35% (Hau, 2008). In contrast, the average lean meat percentage of Ban barrows (n = 45) at live weights ranging from 10 to 30 kg amounted to an acceptable average of 42%, but still a substantial share of these pigs did not fully meet the requirements of the high-end sector (Muth et al., 2015a). This situation demands the cautious balance of trade-offs between product quantity and quality through the collection of data, their integration into feedback loops, and, finally, their transfer into commonly accepted standards. Thus, in paper IV, as a first step, the options for the development of a marketing grid in order to improve the economic profitability of the short value chain for Ban pork, considering carcass and meat quality aspects, were investigated.

#### **6.4.1 Positioning of Ban products derived from a resource-driven production system on the pork market of northern Vietnam**

The comparative study documented in paper III was designed with the objective of investigating whether pork quality and color from the local Vietnamese Ban pig breed can be effectively discriminated from commercial pork from crossbred fatteners, and, thus, whether Ban pork can be positioned on the market and protected from competition by particular quality traits. Thereby, meat quality traits of fresh pork from 6 to 7 month old Ban barrows (n = 33) were compared to those of commercial crossbreds sired by Piétrain x Duroc boars (n = 12) at an equal age. Both genetic groups were reared by smallholders, but according to different objectives and within different production systems (see paper III, chapter 4.2). Slaughter was conducted in mixed groups according to common practice in small-scale abattoirs in order to, as a first step, evaluate the differences between the products as they enter the market. Against this background, traditional Ban pork was clearly distinguishable from commercial pork by a few, yet prominent, features. The size and meatiness of the loin of the Ban pigs was significantly reduced. Furthermore, the thickness of the dorsal fat layer and the marbling content of the longissimus thoracis et lumborum and semimembranosus muscles of purebred Ban pigs were significantly decreased compared to commercial crossbreds. Also, the fatty acid pattern of the longissimus thoracis et lumborum muscle differed significantly between both genetic groups, whereas the proportion of polyunsaturated fatty acids relative to total lipids was significantly increased for the longissimus thoracis et lumborum of Ban fatteners at the expense of

monounsaturated fatty acids (paper III). The reduced cut size and the lower intramuscular fat content are two criteria that strongly relate to the appearance of the product, and could enable the consumer to visually discriminate less marbled Ban pork from commercial pork. The reduced intramuscular fat content could furthermore result in an altered eating quality and act as a credence attribute, communicating – along with the elevated ratio of polyunsaturated fatty acids relative to saturated fatty acids (paper III) – a healthier product. It has, however, to be clearly stated that these last two assumptions are controversial (see also paper III, chapter 4.4).

Due to the fact that the commercial pigs had 25% Piétrain and 25% Duroc inheritance, and were reared under semi-intensive conditions, the considerably increased growth rate and meatiness compared to purebred Ban pigs was not surprising. But, despite that, most pork quality traits were not substantially affected by the genetic group. In a proteomic study, Kwasiborski et al. (2008) showed that meat quality differences were related to alterations in biochemical processes, which varied according to sex and sire breed and, to a lesser extent, also to the rearing environment. However, the common underlying mechanisms were mostly similar, for instance, proteins retained in the models for the ultimate pH, lightness and water holding capacity were often related to the glycolytic pathway, phosphate transfer, or myofiber type composition (Kwasiborski et al., 2008). These findings are in line with observations made by Chang et al. (2003) and Gil et al. (2008), who attributed some of the variation in meat quality to the composition and metabolic profiles of porcine muscles. Assuming a considerable difference in the myofiber type composition between both genetic groups due to the contrasting selection history, one could expect substantial variation in postmortem metabolism and in meat quality between purebred Ban pigs and commercial crossbreds. But, some of the most important physico-chemical aspects of meat, such as the extent of postmortem glycolysis and water holding capacity, only differed slightly between both genetic groups (paper III). With respect to the instrumental color values, the genotypic differences depended on the muscle under investigation, and were only significant for selected color values of the semimembranosus, a muscle of the hindleg, which has been discussed further in chapter 6.3.

In order to compare the incidence of meat defects in both genetic groups, different parameters and threshold values according to Mörlein et al. (2007) were applied, resulting in some contradictory outcomes (Muth et al., 2015b). Setting a threshold of pH 6.0 of loin at 45 min postmortem, the frequency of carcasses from commercial crossbreds classified as being at risk of developing PSE was significantly increased compared to those from purebred Ban hogs (50 vs. 12%, respectively;  $P = 0.013$ ). Hardly any PSE risk was detected when the threshold was reduced to pH 5.8 at 45 min postmortem. Applying drip loss with a maximum of 7% weight loss at 72 h postmortem as a criterion, more Ban carcasses (33%) than carcasses from commercial crossbreds (8%) were classified as being prone to develop the PSE condition, although no statistical significance was reached ( $P = 0.136$ ). Thus, according to one criterion crossbred carcasses appeared to be more at risk to develop PSE than Ban carcasses, whereas according to the other criterion the opposite was observed (Muth et al., 2015b). A similarly paradox situation was reported by Mörlein et al. (2007) for modern crosses; however, the cause could not be identified. The marginal differences in early postmortem glycolysis between the commercial crossbreds and Ban hogs detected (paper III; Muth et al., 2015b) could relate to either a genotypic-specific (physiologic) response to slaughter conditions (Terlouw, 2005), or an effect of diverging live weights. As demonstrated for Ban pigs in paper IV, live weight exerted an effect on pH decline under the present slaughter conditions, possibly due to the elevated ability of bigger pigs to struggle at the fixation of their legs before slaughter. However, based on the

acceptability benchmarks for ultimate pH (5.5 to 6.0) and subjective meat color (score 2 to 4) of fresh loin cuts for different farming systems in Europe (Gonzàles et al., 2014), neither Ban pork, which, on average, exhibited an ultimate pH of 5.6 and a color score of 3.1, nor pork derived from commercial crossbreds, which, on average, exhibited an ultimate pH of 5.6 and a color score of 3.7, was objectionable. Yet, DFD cases were sporadically noted for both genetic groups (Muth et al., 2015b), which could be related to the lairage conditions present in small-scale abattoirs, whereas, in some cases, no pens were available and pigs were tethered. Under these conditions, feed deprivation and exploitation of the glycogen stores can result in the DFD condition (Monin, 2004; Terlouw, 2005). Overall, both indigenous and commercial pork produced by smallholders in northern Vietnam appear to be of acceptable quality. An improvement of slaughter conditions could further reduce the risk of developing meat defects such as PSE and DFD. With respect to the main goal of effectively discriminating Ban pork from commercial produce, most physico-chemical meat quality parameters do not seem to be appropriate because they are quite similar between both genetic groups. In contrast, the lipid characteristics of the carcass and meat revealed some remarkable differences between purebred Ban fatteners and commercial crossbred pigs sired by Piétrain x Duroc boars, which are discussed in the following section.

The decreased absolute and relative quantity of lipid deposits (i.e. carcass fat,<sup>15</sup> subcutaneous fat, and intramuscular fat) of purebred Ban pigs compared to commercial crossbreds, and the altered quality of the intramuscular lipid deposit most probably resulted from an interaction between genotype, slaughter weight and the production system. In paper IV, it was demonstrated that – under the given feeding regime – the subcutaneous and carcass fat deposits of Ban pigs increased for pigs weighing less than 12 kg compared to those weighing more, but then remained constant for a span of live weights up to around 20 kg. At a body weight of approximately 20 kg, the fat deposits of the Ban pigs amounted to an average of 14% relative to the carcass weight, which is only slightly increased when compared to those reported for intensively reared Piétrain-sired crossbreds at a similar live weight (Landgraf et al., 2006). As demonstrated in paper IV (chapter 5.4), the development of some carcass measurements and the properties of the carcass composition of Ban pigs from 12 to approximately 20 kg also resembled that of modern pig breeds and commercial crosses. For instance, the lean meat weight increased while the lean meat percentage remained constant, which is similar to the growing phase – obviously at a higher level in body weight – of genetically selected genotypes (Martin et al., 1980; Gu et al., 1992). However, while the relative proportion of carcass deposits in modern crosses were maintained up to a live weight of 60 kg and beyond (Landgraf et al., 2006), the study by Hau (2008) showed that at a live weight of 50 kg, the carcass fat in Ban porkers already amounted to an average of 41%, and the depth of subcutaneous fat to 30 mm, whilst the lean meat percentage reached an average of only 35%. Thus, similar to slow-growing local breeds of southern Europe, such as the Iberian pig (Nieto et al., 2013), fat accumulation in Ban porkers is initiated at a lower body weight compared to modern breeds. The determination of the optimum slaughter age and weight before the fat deposits exceed the acceptance threshold is therefore crucial. Possibly, cautiousness with respect to the features of the product resulted in the suboptimal low body weight requested by the retailers surveyed by Huyen et al. (forthcoming). Thus,

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<sup>15</sup> The difference in carcass fat between Ban pigs and commercial crossbreds sired by Piétrain x Duroc have not been published yet. The mean value and standard deviation for the carcass fatness of Ban pigs of the data set analyzed for paper III amounted to  $10.4 \pm 8.1\%$  ( $n = 32$ ), whereas the mean value and standard deviation for commercial crossbreds amounted to  $16.5 \pm 5.1\%$  ( $n = 12$ ). Note: also compare to the results of paper IV.

in contrast to European autochthonous breeds, for which elevated fat deposits compared to conventional genotypes are accepted in order to produce specialty products, the fat deposition in Ban carcasses and pork dedicated for high-end markets was reduced compared to the commercial product offered in northern Vietnam (see paper III, chapter 4.3), even though a genetic potential for excessive fat deposition appears to exist (Hau, 2008). One cause for the discrepancy in the development of lipid deposits of Ban pigs compared to commercial crossbreds could be founded in the lagged initiation of fat deposition of Ban barrows due to a late maturation of the breed, but there are some indications that further genetic and environmental factors substantially contributed to the retarded expression of fat deposits.

Clearly, there is a genetic determination of lipid deposition in pigs with heritability values of 0.31 for dorsal backfat depth (Clutter, 2011), and 0.26-0.86 for intramuscular fat concentration (Ciobanu et al., 2011). When slaughtered at the same chronological age, Jinhua pigs exhibited significantly increased levels of intramuscular fat in the longissimus muscle at the ages of 90, 120 and 150 days compared to Landrace pigs (Wu et al., 2013). It was shown that for the former the transcription of genes encoding for the fatty acid biosynthesis and for myostatin as inhibitor for myogenesis were up-regulated (Wu et al., 2013). Also, differences in early adipogenesis could account for genetic variation in intramuscular lipid deposition (Liu et al., 2009). The Ban breed has been subjected to natural and/or unconscious selection for the last centuries, but has not been selected against backfat thickness. Therefore, supported by the results of Hau (2008), it can be assumed that the genetic potential is high for fatty acid biosynthesis and fat deposition, but low for lean growth. At low body weights under the given husbandry system, this genetic potential is not expressed despite the relatively high slaughter age of 180 to 210 days as applied in the present study. In order to elucidate the reason for this, the effect of the rearing environment has to be considered. Some extensive production systems in southern Europe exhibit features that roughly correspond to the resource-driven Ban husbandry system described in paper III. In the sylvo-pastoral systems of southwestern Europe, rearing is carried out outdoors and feeding is based partly or exclusively on natural resources (Pugliese and Sirtori, 2012). In these systems, phases of restricted feed availability followed by availability of starch-rich (i.e. energy-rich) acorns may elicit compensatory growth, i.e. a “catch-up” phase of accelerated weight gain under improved nutrition, which particularly concerns the lipid tissue (Lebret, 2008; Pugliese and Sirtori, 2012). Ban pigs intended for the high-end niche market obviously lack a phase of compensatory growth; therefore, the emphasis of the following is focused on the phase of feed restriction. It is well-established that where the nutrient intake required for lean deposition is not met, fat deposition is not initiated (Clutter, 2011). Unlike the southern European production systems for high quality pork, Ban pigs are removed from the system before the nutrient intake is sufficient to meet the demands for lean growth and/or lipid deposition. The highly fibrous diet fed to Ban pigs probably represented a de facto feed restriction. Gondret and Lebret (2007) showed that after a phase of feed restriction (70% of *ad libitum* intake), the growth rate, the subcutaneous fat layer, and the lipid content of both the rhomboideus and biceps femoris muscle of Duroc x (Large White x Landrace) crossbreds were reduced in comparison with *ad libitum* fed pigs. Similar results, i.e. a reduction in carcass weight and backfat thickness subsequent to feed restriction, were obtained by Skiba et al. (2012) for Danish Landrace x Large White crossbreds; but, in contrast to the results of Gondret and Lebret (2007), the intramuscular fat content in the longissimus dorsi of restricted pigs was not reduced. In addition, the nutrient deficient feed for Ban fatteners possibly contributed to an altered fatty acid pattern of intramuscular lipids, which differed from commercial crossbreds through elevated proportions of polyunsaturated fatty acids – mainly linoleic acid –,

whereas the proportion of monounsaturated fatty acids – predominantly oleic acid – was reduced. Phases comprised of a 25% reduced feed intake induced an increase in the proportion of polyunsaturated fatty acids relative to the proportion of saturated fatty acids in growing Duroc x (Polish Landrace x Polish Large White) pigs (Więcek et al., 2011). Accordingly, a wide ratio of the proportion of polyunsaturated fatty acids to the proportion of saturated fatty acids was observed for Ban fatteners. However, the proportions of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids as such were not influenced in the trial by Więcek et al. (2011). Additionally, besides the nutrient deficient diets, the access to free range or spacious enclosures could increase further the nutritional requirements due to the elevated physical activity of scavenging animals (Thomas et al., 2013), contributing more to the limited lean growth and restricted development of lipid deposits of Ban pigs. It could be speculated that if smallholders were to permanently pen the animals and augment the energy level in diets, lean growth could be improved, but accompanied by a much earlier fat deposition, including the replenishment of intramuscular fat deposits. It remains questionable whether the product derived from such a feeding regimen would still exhibit the features that are currently appreciated by the market. It would therefore be necessary to investigate how far the current management and feeding practices safeguard the desired attributes of specialty Ban pork compared to the merits of accelerated lean growth in (semi-) intensive husbandry systems. Such research could result in the development of rearing protocols that take the feed resources and artisanal production techniques of smallholders into account, and link them to the product requirements of retailers. Thereby, binding protocols could represent an additional barrier that protects the Ban specialty pork niche market from being taken over by competitors.

The ability to distinguish Ban pork from commercial produce by lipid characteristics must also be approached by considering a second aspect. According to Toften and Hammervoll (2013), a niche product requires a mainstream commodity as a reference. This indicates that the frame of reference for commodity pork must be adjusted to the country-specific conditions. It is noteworthy that, in northern Vietnam, and probably also in other countries where the industrial pig production system is not yet fully implemented, commercial pork appears to contain more (intramuscular) fat compared to commodity pork from large-scale industrial production. In fact, the intramuscular lipid content in pork from semi-intensively reared commercial crossbreds amounted to an average of 3.3% in the longissimus thoracis et lumborum, and to 3.1% in the semimembranosus (paper III). In line with this finding, Luc et al. (2014) reported that the intramuscular fat content from the loin in crossbred progeny from Mong Cai x Large White dams mated to either Duroc or Piétrain boars amounted to a similar average of 2.8 to 2.9%. In their study, the porkers were also produced by smallholders for the commodity market in the northern Vietnamese lowlands (Luc et al., 2014). The reduced intramuscular fat content typically observed for conventional genotypes can be explained by the long-term selection against backfat thickness and its (loose) genetic correlation with carcass fatness and intramuscular lipid concentration (Suzuki et al., 2005; Ciobanu et al., 2011; Ros-Freixedes et al., 2013). In relation to figures typical for commodity pork from hybrid pigs produced under optimized feeding conditions (1 to 2% intramuscular lipid content; Ciobanu et al., 2011), the results presented in paper III and by Luc et al. (2014) imply that the reference for commercial pork in northern Vietnam is represented by augmented dorsal fat depths and substantial intramuscular lipid contents. The high intramuscular fat content of commercial crossbreds in the present study is most probably related to the fact that the market-oriented smallholders also incorporated local breeds on the dam side – mainly because of their adaptability to a broad feed spectrum (Rößler et al., 2009) – and to the diets

fed. The diets provided by market-oriented smallholders might have been increased in energy content compared to those of resource-driven households because the rations were corn-based, but probably protein-deficient (paper III). Possibly economic considerations drove farmers to rely on cheap and energy-rich but protein- and/or lysine deficient rations instead of purchasing compound feed. The feeding data presented in paper III, however, was questionnaire-based and not cross-checked, but confirmed earlier findings of Lemke et al. (2006) reporting similar feeding conditions for small-scale yet market-oriented pig producers in Son La, northwestern Vietnam. Pham et al. (2010) reported a comparable diet composition provided by lowland, semi-intensive farmers in Central Vietnam, except that maize was substituted by rice as a main feed component. Thus, by assuming slightly protein deficient diets on semi-intensive feeding levels by market-oriented smallholders in northern Vietnam, the intramuscular fat concentration in muscles of pigs could be increased by stimulating the expression of SCD1 (stearoyl-CoA desaturase-1), which catalyzes the cellular biosynthesis of monounsaturated fatty acids (Hocquette et al., 2010). The high proportion of oleic acid in the intramuscular fat of commercial crossbreeds supports this hypothesis (paper III). Danish Landrace x Large White crossbred gilts subjected to protein restriction showed a decreased carcass weight, but backfat thickness, leanness and intramuscular lipid content were not affected compared to a control. However, interestingly, the group which was restricted in dietary protein exhibited a decreased proportion of polyunsaturated fatty acids in intramuscular fat compared to the control (Skiba et al., 2012). On the other hand, the proportion of monounsaturated fatty acids did not differ from the control group. In addition, the ratio of polyunsaturated fatty acids to saturated fatty acids was significantly decreased for pigs fed on a subtle protein deficient diet (0.29) compared to an experimental group which was restricted in feed intake and a control group (0.40-0.45; Skiba et al., 2012).

Overall, protein-deficient diets and possibly the contribution of local breeds on the dam side may have contributed to the relatively fattier carcasses and pork, and to the high monounsaturated fat proportion in the intramuscular fat of the commercial crossbreeds produced by smallholders in the present study. It must be highlighted that, in northern Vietnam, these small-scale farm types supply the bulk of commodity pork available on markets (Lapar et al., 2012). Thus, as long as the bulk of commodity pork is produced on a small-scale level, incorporating local breeds on the maternal side and nutritionally imbalanced diets, the discrimination of Ban pork from commodity pork by its specific lipid characteristics is facilitated. In contrast, the other physico-chemical parameters of Ban pork are rather similar compared to commodity pork. The intramuscular lipid content and the fatty acid pattern of specialty Ban pork is also quite different from that of rustic European breeds (Pugliese and Sirtori, 2012), and, because of the absence of a compensatory growth phase, actually more akin to pork from lean genotypes stemming from industrial production systems (see paper III, chapter 4.4). If the Vietnamese pork market were to be restructured and large-scale enterprises were to gain considerably more market share than the maximum 12% projected for 2022 by Lapar et al. (2012) the frame of reference could be changed, and the Ban specialty pig could lose its peculiarity with regard to intramuscular fat content if the level of this trait is reduced for industrially produced pork.

#### **6.4.2 Can elevating Ban pig slaughter weight improve the economic outcome with consideration of the strict market requirements of the high-end specialty market?**

In order to establish an alternative food network for Ban pork, it is crucial to develop a normative framework (see chapter 1.1) that also accounts for the optimization of the economic profitability of the stakeholders participating in the short value chain. As previously mentioned, Ban husbandry is

rather a sideline activity of remote smallholders with poor access to markets. But, in contrast to semi-intensive producers, the economic outcome of resource-driven production of purebred Ban pigs was hardly affected by the volatility of feed prices, according to the projections by Lemke and Valle Zárate (2008). This lowers the production risk in case acceptable output prices cannot be achieved, however, at the same time, the gross margins and net benefits realized by this low-input low-output system are marginal. Lemke and Valle Zárate (2008) predicted that the resource-driven production of purebred Ban can be economically successful in cases where the output prices per unit live weight would increase by 92% from their baseline scenario. Huyen et al. (forthcoming) showed that by connecting farmers to the niche markets of Hanoi, a rise of output prices in this magnitude could be achievable. Due to the need for almost zero inputs, particularly in terms of feed, the production system of indigenous Ban pigs by smallholders in northwestern Vietnam is not comparable to the situation of specialty pig producers in Europe and the United States, where high quality pork production tends to increase costs because of, for instance, a poor feed conversion of local breeds (Hueth et al., 2007; Bonneau and Lebret, 2010).

The findings of a marketing study analyzing data of the sales of 107 Ban pigs showed that smallholders realized a considerable mark-up per unit of live weight relative to sale on the regional market, but the producers had to sell finishers at low market weights in order to comply with retailer requirements (Huyen et al., forthcoming). The quantity of meat output per sow is, therefore, considerably reduced when high-end niche markets are supplied, and this situation must be balanced against the use of traditional outlets that accept high body weights. But, as long as excessive lipid deposition is not triggered and the quality of fresh pork can be maintained, a moderate increase in the slaughter weights from the current market weights could improve the economic outcome for primary producers as well as downstream actors of the value chain. Paper IV was, therefore, dedicated to pursuing the question of whether a marketing grid favoring slightly increased slaughter weights could improve the economic profitability of the short value chain for Ban pork, in strict accordance with the carcass and meat quality requirements imposed by the urban high-end market of northern Vietnam. Additionally, the potential effects on livelihoods of smallholder farmers in northwestern Vietnam were discussed in this chapter.

In paper IV, live weight classes were formed from a sample of Ban barrows ( $n = 51$ ) dedicated for specialty pork marketing. The live weight category of 12 to 15 kg, which encompasses the currently predominant market weight for Ban pigs for the urban specialty market (Huyen et al., forthcoming), neither differed in carcass fatness nor in dorsal fat thickness compared to pigs weighing slightly more than 18 kg (paper IV). Likewise, the lean meat percentage was not altered, but oscillated around 40% among live weight categories. Thus, moderately increasing the slaughter weight had no adverse effects on carcass quality, while the carcass and lean meat weight output was significantly increased. It is assumed that somewhere beyond a live weight of 20 to 30 kg – the approximate threshold could not be determined in the present study –, a pronounced lipid accumulation in the carcass takes place, resulting in the highly adipose carcasses of heavier Ban pigs recorded by Hau (2008). Besides carcass quality, the meat quality should also not be compromised when elevating the slaughter weight, because a deterioration of meat quality could threaten the market position of Ban pork as high quality product. Indeed, paper IV showed that the early postmortem glycolysis of Ban barrows could be adversely affected by increasing the slaughter weight. The trend for pH recorded at 45 min postmortem was linearly negative, and could have reached a situation in which a larger proportion of Ban carcasses could have been prone to develop the PSE defect (paper IV). The cause is not clear, but



given that slaughter is carried out manually without prior stunning, stressful conditions around slaughter could interact with the live weight of Ban pigs and their ability to struggle. Therefore, further studies are required to optimize the slaughter process for slightly heavier Ban porkers. The association between slaughter weight and postmortem glycolysis was, however, only noted for the longissimus thoracis et lumborum muscle, but not for the semimembranosus. Except for a tendency of increased cooking losses of the longissimus thoracis et lumborum, no other negative impact of an increase in slaughter weight on meat color or quality trait was noticed. According to the findings of paper IV, the intramuscular lipid content was not altered by increasing the live weight from below 10 to up to 30 kg. Thus, as shown in paper IV, under the consideration of carcass and meat quality characteristics, a moderate increase in live weight to around 20 kg at slaughter could be feasible. In order to translate this into practice, a marketing grid, accepted by both smallholder producers and downstream actors of the value chain, must be developed.

The set-up of a marketing grid requires the application of attributes suitable for the monitoring and verification of the quality of the produce and, eventually, the quality-based remuneration of producers. The simplest form would be a live weight-based grid. However, considerable animal-to-animal variation in lean and particularly carcass fat percentage within live weight categories indicates that a quality-based evaluation of carcasses, according to slaughter weight, might not be sufficiently accurate (paper IV). For this, as a first approach, a method for lean meat percentage estimation in Ban carcasses based on the “two point” measurement was applied, and, finally, the equation according to the Commission Decision 96/4/EC was fitted (Muth et al., 2015a). But, the high root mean square error of prediction (4.5%) indicated that this method requires recalibration before a routine application for Ban fatteners slaughtered in contracted small abattoirs is realistic. This method and other measures for quality control, such as a measurement of backfat thickness, seem to be applicable only in the midterm in relation to the implementation of a further value adding “package”, for instance, the development of a brand for indigenous Ban pork. Thus, at least in the current phase, the grid must be, by default, based on the live weights of marketed Ban pigs. In order to enhance the attractiveness for buyers to opt for heavier Ban pigs, two degressive pricing systems were suggested in paper IV. These pricing systems mimic the actual price formation at farm-gate; therefore, a mutual acceptance of the degressive set-up by the stakeholders is assumed. Because the dressing percentage and carcass quality of Ban barrows was not altered when the live weight was increased to up to around 20 kg, both producers and retailers could benefit from the implementation of these systems. The decline in commodity prices per unit of carcass and lean are demonstrated in paper IV, indicating that clear cost benefits for the retailers could be achieved. The economic outcome for retailers could be even further improved by declined transaction costs per weight unit at increased slaughter weights. García-Macías et al. (1996) reported that economic yield varied according to the genetic makeup of the crossbreds in their study, mainly due to differences in the distribution of cut weights. In contrast to the presented data, the live weight (90 vs. 120 kg) did not influence economic yield in their study. But, García-Macías et al. (1996) utilized a fixed price per unit of carcass as a basis for their calculation, as opposed to the degressive system applied herein. In addition to the benefits for downstream actors by declined commodity prices, the application of a degressive pricing system, along with an increase in market weight of up to around 20 kg, would result in a rise of producers’ revenues of approximately 380,000 to 420,000 Vietnamese Dong per pig sold relative to the price obtained for a pig weighing 12 to 15 kg (+37%). The poverty line of Vietnam has been set to 435,000 Vietnamese Dong per person and month, and, in the northwestern mountainous region, 60% of households fall below this threshold (World Bank, 2012), highlighting

the relevance of this slight increase in revenue. Establishing a value chain that allows the smallholders of remote regions to sell a part of their Ban pigs at a premium, combined with a grid which ensures optimum conditions for both contracting parties, could thus contribute to the overall goal of improving livelihood conditions of smallholders through diversified incomes. The results from these economic considerations, supported by the data of outlets and marketing strategies, and expanded by information on the product and the options of compliance with quality requirements (papers III and IV), further support the recognition of specialty pork production as an additional source of income for remote smallholder producers in northern Vietnam.

Still, the question of whether the specialty Ban pork value chain brings such proposed benefits to farmers and society has yet to be proven, and some skepticism may be appropriate. The actual demand could not be quantified in the marketing study carried out in the same project (Huyen et al., forthcoming). Thus, it could be the case that the market for specialty pork – as was noted for the market for local food products in the Scottish/English border region (Ilbery and Maye, 2005) – could be quickly saturated and prices become too low to maintain the value chain. Ban pork is rather a luxury good, and the demand for this product is income elastic. With less income available, such as during the economic crisis of 2008, the demand for Ban pork is therefore expected to fall. But, as long as the low-input production practices of Ban keeping are maintained, and rural outlets, as well as usage alternatives, are available, a fall in demand would not result in an economic threat for farmers. In addition, free-riders could threaten the stability of the markets (van der Ploeg et al., 2012; Phuong et al., 2014). Therefore, linking a quality assurance scheme to a trademark or a special label for Ban pork, based on selected carcass and meat quality criteria, could contribute to consistency in the quality produced and safeguard stable communication channels among stakeholders in the value chain, as well as support the build-up of a reputation for a trustworthy producer-customer relationship (FAO, 2013). In this respect, a normative framework representing a common pool resource could be constructed around the value chain for Ban specialty pork, protecting the network from competition and market failure. Besides practices aimed at the maintenance of the distinctiveness of the products, this framework needs to incorporate technical and economic parameters that optimize the profitability of the diverse stakeholders of the value chain. At present, the short value chain cannot be coordinated by the cooperative farmers alone without support through research, extension and policy measures. But, in the case that the requisites required for infrastructure and governance are met, sustainable economic utilization of the Ban breed for the benefit of marginalized smallholders in northern Vietnam seems possible. The options for outscaling to other northern and central mountainous provinces in Vietnam, as well as to other similar eco-cultural regions of the Southeast Asian Massif, e.g., in Laos, China and Thailand, should be evaluated in the near future.

## 6.5 General conclusions

In view of the emerging alternative food networks and the desire for *in situ* conservation of local pig and chicken breeds, the genotype-specific differentiation of product quality became an important subject of research. Grunert (2005, pp. 371-372) pointed out that one of the main challenges in food production is to “translate consumer wishes into physical product characteristics, and only when consumers can then infer desired quality from the way the product has been built, will quality be a competitive parameter”. Therefore, much effort has been placed on generating detailed knowledge and documentation of the breed- and genotype-specific attributes of products, and of the factors exerting an influence on these parameters. In meat research, these efforts have been accompanied by controversy as to what extent selection on growth performance affects meat color and quality. With respect to this topic, the present work provides a differentiated picture.

In this thesis, two approaches, one on-station experiment on slow- and fast-growing chicken lines and one applied research project involving a native pig breed of northern Vietnam, were implemented to investigate the genotypic differences in the meat color and quality of pig and chicken genetic resources. These differences can, assuming that they relate to sensory characteristics, result in a distinctive appearance and eating quality of the products, and an exceptional positioning on the market.

The differences in growth and carcass traits among each of the pig and chicken genotypes were substantial, but, in contrast, the variation in some of the most prominent physico-chemical quality parameters was marginal. It was assumed that standardization according to chronological age contributed to the similarity in meat quality parameters, while in other works standardization according to live weight at slaughter might have resulted in pronounced age-related effects. The hypothesis that selecting for growth and carcass traits relates to deterioration of meat color and quality, and particularly to a decline in the heme pigment content, was not supported by this research. This indicates that meat quality can be genetically improved independently from growth performance.

The increased cooking loss of the fast-growing commercial broilers compared to both slow-growing experimental chicken lines in the on-station experiment appeared to constitute an exception because, irrespective of the slaughter age, cooking losses increased for the broilers. Effects on sensory quality could not be inferred from this work, but an economic loss through reduced yields seems to be unavoidable. This aberration in breast meat functionality under heat exposure is a feature that was recently attributed to an emerging defect in contemporary broiler flocks: the white striping condition. Based on the present data, a relationship between growth rate and cooking loss cannot be excluded, but this association seems to be rather complex and non-linear because within the slow-growing experimental chicken lines a favorable correlation of residuals between cooking loss and maximum daily growth was detected. This hypothesis is a unique outcome of this thesis; however, it remains to be verified by follow-up research. The advantage of slow-growing lines in regard to cooking yield over contemporary broilers may only be a brief phenomenon, depending on the ability of the broiler breeding industry to respond to the issue. Distinct chicken meat products within alternative production systems could be moreover obtained by extending the fattening period because several physico-chemical traits depended on the age at slaughter, particularly the yellowness of breast meat and its shear force.

For an indigenous pig breed of northwestern Vietnam – the Ban –, the inevitable connection of the breed with a specific production system, described in paper III, chapter 4.2, resulted in a product that was clearly distinguishable from the commercial product by its reduced carcass fatness, subcutaneous fat depth, and lipid concentration in the loin and ham muscles. These practices should be maintained when aiming at a consistent product quality. This is noteworthy as it is assumed that the genetic potential of this local breed for fat accumulation is increased when compared to commercial crossbreds, but not expressed due to the particular way of rearing, feeding and timing of slaughter. The discriminability of Ban pork by its marbling content was also enabled through the relatively high intramuscular fat content in the muscles of commercial crossbreds, which was attributed to the utilization of local breeds on the dam side and to the use of protein deficient diets. Thus, similar to the situation proposed for chickens before, the discriminability of Ban pork depends to a certain degree on the dynamics present in the commodity sector. Although Ban producers could obtain a mark-up per weight unit of their product by addressing urban high-end markets, they had to accept considerably lower live weights of specialty Ban porkers at marketing. To balance this situation out, a marketing and pricing system was proposed, allowing for a slight increase of the current slaughter weights for specialty Ban pigs in order to augment the economic benefits generated by the value chain without compromising the carcass and meat quality. The data on product quality and the optimization of target weights could support the build-up of one of the first formalized value chains for specialty Ban pork in Vietnam, contributing to the sustainable use of the Ban breed for the benefit of a marginalized smallholder farmer community.

In summary, this thesis indicates that the application of non-conventional and/or slow-growing genotypes alone does not guarantee a superior and/or distinctive meat color and quality of the marketable products. It was rather the case that, in line with the statement of Pugliese and Sirtori (2012) on traditional European pig husbandry systems, the uniqueness of meat products is critically dependent on the match of the respective genotype with appropriate production methods. Thus, for instance, a discerning choice regarding slaughter age and feeding strategy. Additional cornerstones necessary for the development of a sustainable niche market for high quality monogastric meats, such as cost-benefits analyses and adequate distribution modes and governance structures, have to be addressed as well.

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## 7 Summary

In response to the restructuring of the meat sectors, globalization of agro-food chains, and increasingly differentiated consumer needs, alternative food networks focused on the production of high quality pig and chicken meat have emerged in rural areas. Some of these initiatives base their production and marketing concepts on traditional pig and chicken breeds, and, thus, on animals that markedly diverge from conventional genotypes in terms of genetic background and, in particular, meat production efficiency. Against this background, this thesis investigates two main areas: how far genotypic differences and growth performance could be related to a distinctive meat color and quality; and how production and marketing strategies could be optimized in order to serve niche markets. For this, two contrasting approaches on pigs and chickens were taken.

Firstly, in an on-station experiment, male birds ( $n = 264$ ) of three chicken lines – two slow-growing experimental lines and a fast-growing commercial broiler – were compared under standardized environmental conditions, testing the hypothesis that the application of slow-growing genotypes in chicken meat production systems provides a distinctive meat color and quality. The lines were slaughtered at an equal age (7 and 10 weeks), and, additionally, a fast-growing broiler group was slaughtered at the age of 4 weeks to allow for a comparison of genotypes at a similar body weight. The results showed that the expression of most meat color and quality traits depended on the actual slaughter age/weight in a genotype-specific manner. The differences in the heme pigment concentration among chicken lines were marginal, and, although hemoprotein content contributed to the redness of breast meat, did not translate into a largely diverging meat color. Taking the results from the pig trial additionally into account, the hypothesis that selecting for growth and carcass traits relates to a decline in the concentration of heme pigment and reduced instrumental redness of monogastric meats could not be supported. The pH of breast meat measured at 24 h postmortem depended on the slaughter age of the broiler relative to the point in time when the maximum daily weight gain was achieved, pointing toward an association between the expression of pH and myofiber hypertrophy. A clear effect of the chicken line on the ability of the meat to retain liquids under thermal exposure was revealed, irrespective of the age at slaughter. The cooking loss was clearly compromised for the commercial broiler as meat samples exhibited a relative weight loss of 8 to 13 percent points more than the slow-growing lines. The breast meat functionality of broilers, particularly of those birds slaughtered at 7 and 10 weeks of age, resembled the “white striping” condition, a meat defect occurring in contemporary broiler flocks. Thus, despite the strong indication that the water holding capacity of breast meat during cooking taken from fast-growing broilers is compromised compared to the slow-growing chickens, the conclusion that selection on growth performance would deteriorate meat quality *per se* could not be drawn. Indeed, complex non-linear relationships of pH and cooking loss with growth rate were implied by the within-line analysis of residual correlations of meat quality traits with growth curve parameters. In summary, effects of genotype on chicken meat quality exist, but factoring out the effect major genes it appears that they can be effectively exploited only in combination with the appropriate production methods and timing of slaughter.

Secondly, an explanatory-observational study embedded in a project that aimed at the establishment of a short supply chain for pork from the native “Ban” breed in northwestern Vietnam was conducted. Here, the meat color and quality of Ban barrows ( $n = 33$ ) intended for marketing at urban high-end markets were compared to that of commercial crossbreds ( $n = 12$ ) sired by Piétrain x Duroc boars. When reared in their respective production systems, divergent growth and carcass

performances were noted, but most of the physico-chemical meat quality parameters, including meat color and the myoglobin concentration, did not essentially differ between both genetic groups. However, the lipid characteristics clearly varied between Ban porkers and commercial hogs. Notably, the intramuscular fat percentage in the loin and ham of Ban fatteners was significantly reduced compared to that of commercial crosses. Additionally, the fatty acid pattern in the loin differed; whereas the significantly higher proportion of linoleic acid of traditionally produced pigs could possibly result in a preferred nutritional value, but lower oxidative stability of the products. Probably, the low nutritional density of the diets of Ban barrows, which were based on rice bran and banana pseudostem, could have limited their ability to exploit their genetic potential for lipid deposition. Thus, lipid characteristics in particular allow for a discrimination of specialty Ban meat from commodity pork produced in northern Vietnam and its positioning on the market. Furthermore, the data of Ban fatteners slaughtered for the high-end market ( $n = 51$ ) indicated that the current marketing weights could be economically suboptimal, and a slight increase in marketing weight from the current weight of between 12 and 15 kg to up to approximately 20 kg under the given husbandry conditions is feasible without hurting the quality of the carcass or pork. The economic success of stakeholders along the value chain could thus be improved by implementing a marketing grid based on a degressive pricing system that could support the build-up of one of the first formalized food networks for high-quality Ban pork in Vietnam.

In summary, this thesis indicates that the application of non-conventional and/or slow-growing pig and chicken genotypes alone does not guarantee a superior and/or distinctive meat color and quality of the products. It appears that the valorization of monogastric meat products through niche marketing is critically dependent on matching the respective genotype with appropriate production methods, thus, for instance, highlighting the need for producers to be discerning in regard to the choice of slaughter age and feeding strategy. In the case that these premises are implemented, a distinguished positioning of meat products derived from local pig and chicken genetic resources can be realized, and, expanded by standards that also take the social and economic dimensions of food value chains into account, translated into a common pool resource as a basis for an alternative food network. Efforts should then be directed toward the elaboration of stable distribution channels and effective governance structures in the respective food network.

## 8 Zusammenfassung

Infolge der Restrukturierung der Schweine- und Geflügelfleischsektoren, der Globalisierung der Wertschöpfungsketten für Lebensmittel und der parallel dazu verlaufenden Ausdifferenzierung der Konsumentenbedürfnisse entwickelten sich im ländlichen Raum alternative Wertschöpfungsnetzwerke für hochqualitative Schweine- und Geflügelfleischprodukte. Die Produktions- und Vermarktungskonzepte einiger dieser Initiativen basieren auf traditionellen Schweine- und Geflügelrassen und somit auf Tieren, welche sich hinsichtlich der Genetik und der Produktionseffizienz deutlich von konventionellen Genotypen unterscheiden. Vor diesem Hintergrund befasste sich diese Arbeit mit zwei Hauptthemen. Zum einen mit der Frage inwieweit Genotypeneffekte sowie die Wachstumsleistung mit der Fleischfarbe und -qualität zusammenhängen und zum anderen wie Produktions- und Vermarktungsstrategien in der Ausrichtung auf Nischenmärkte optimiert werden können. Dieses wurde anhand zweier unterschiedlicher Ansätze mit Bezug auf Schweine bzw. Hühner untersucht.

Zunächst wurden in einem kontrollierten Experiment Hähne ( $n = 264$ ) dreier verschiedener Hühnerlinien – darunter zwei langsam wachsende experimentelle Linien und ein schnell wachsender kommerzieller Broiler – unter standardisierten Umweltbedingungen verglichen. Hierdurch sollte die Hypothese, dass durch den Einsatz langsam wachsender Hühner eine unterscheidbare Fleischqualität erzeugt werden kann, getestet werden. Die Linien wurden dabei jeweils anhand des gleichen Schlachalters (7 und 10 Wochen) sowie auf Basis eines vergleichbaren Schlachtgewichts bewertet, daher wurden schnell wachsende Broiler zusätzlich im Alter von 4 Wochen geschlachtet. Die Ergebnisse zeigten, dass die Ausprägung der Fleischfarbe sowie der meisten Fleischqualitätsmerkmale, jeweils in Abhängigkeit des Genotyps, durch das Schlachalter/-gewicht bestimmt wurde. Obwohl ein Zusammenhang zwischen dem Gehalt an Hämoprotein und des Rotwertes des Fleisches nachgewiesen werden konnte, resultierten die marginalen Unterschiede in der Konzentration an Hämpigmenten zwischen den Hühnerlinien nicht in einer deutlich abweichenden Fleischfarbe. Somit konnte die Hypothese, dass eine Selektion auf Wachstums- und Schlachtkörpermerkmale mit einer Verringerung des Hämpigmentgehalts und des Rotwertes des Fleisches von Monogastriern assoziiert ist, nicht verifiziert werden. Ähnliche Ergebnisse wurden auch aus den Versuchen an Schweinen gewonnen. Der zu einem Zeitpunkt von 24 h nach der Tötung erfasste pH-Wert des Brustfleisches der schnell wachsenden Broiler hing vom Alter bei Schlachtung relativ zum Alter bei maximaler Gewichtszunahme ab, wodurch sich ein Zusammenhang der Ausprägung des pH-Wertes und des radialen Wachstums der Muskelfasern ableiten ließe. Im Gegensatz zu dem deutlichen Einfluss des Alters auf den pH-Wert, wurde für die Kapazität des Fleisches unter Hitzeeinwirkung Flüssigkeit zu binden ein deutlicher, vom Schlachalter unabhängiger, Genotypeneffekt nachgewiesen. Der Kochsaftverlust des Brustfleisches der Broiler war hierbei um 8 bis 13 Prozentpunkte gegenüber dem der langsam wachsenden Linien erhöht. Die funktionellen Charakteristika des Brustfleisches der in der 7. und 10. Lebenswoche geschlachteten Broiler ähnelten daher dem sogenannten „white striping“ Befund, einem Fleischdefekt, welcher derzeit gehäuft in Broilerbeständen auftritt. Trotz der Hinweise auf die erheblich beeinträchtigte Wasserhaltekapazität von Broilerbrustfleisch im Verhältnis zum Fleisch langsam wachsender Hühner, scheint eine Selektion auf Wachstumsleistung nicht *per se* die Fleischqualität zu vermindern. Tatsächlich deutete der Vergleich der jeweils innerhalb der Linien berechneten Residualkorrelationen der Fleischqualitätsmerkmale mit Parametern der Wachstumskurve auf komplexe, nicht-lineare Beziehungen zwischen der Wachstumsrate von Hühnern und dem pH-Wert bzw. dem Kochsaftverlust hin. Zusammenfassend scheinen also durchaus Genotypeneffekte auf die Hühnerfleischqualität zu

existieren, allerdings können diese, lässt man die Effekte von Majorgenen unberücksichtigt, wohl nur durch gezielte Anpassungen der Produktionsweisen und des Schlachtgewichts/-alters genutzt werden.

Eine weitere, explorativ-analytische Studie zur Qualität von Schweinefleisch der Lokalrasse „Ban“ in Nordwestvietnam war in ein Projekt zur Etablierung einer Wertschöpfungskette im Rahmen einer kleinbäuerlichen Zucht- und Vermarktungskoooperative eingebettet. Dabei wurde die Fleischfarbe und -qualität von für die Vermarktung im städtischen Hochpreissegment vorgesehenen Ban-Börge (n = 33) mit kommerziellen Börge (n = 12) verglichen, die aus Kreuzungen mit Piétrain x Duroc-Ebern stammten. Wurden diese Vergleichsgruppen in ihrem jeweiligen Produktionsumfeld erzeugt, so erzielten die Tiere deutlich unterschiedliche Wachstums- und Schlachtkörperergebnisse, wohingegen sich die meisten der physikalisch-chemischen Fleischqualitätsparameter, einschließlich der Fleischfarbe und des Myoglobingehaltes, nicht wesentlich unterschieden. Im Gegensatz dazu variierten die Fettcharakteristika der Ban-Tiere deutlich im Vergleich mit kommerziellen Schlachtschweinen. Bemerkenswerterweise war der intramuskuläre Fettgehalt des Koteletts und des Schinkens der Ban-Mastschweine hierbei signifikant reduziert. Des Weiteren unterschied sich die Lipidfraktion des Koteletts hinsichtlich ihrer Zusammensetzung, wobei der signifikant erhöhte Anteil an Linolsäure der auf traditionelle Weise produzierten Schweine möglicherweise in einem erhöhten Nährwert, aber auch einer verminderten oxidativen Stabilität der Produkte resultierte. Vermutlich trug die geringe Nährstoffdichte der vorwiegend auf Reisspelzen und Bananen-Pseudostamm (*Musa balbisiana*) basierenden Futtermitteln der Ban-Börge dazu bei, dass deren genetisches Potenzial für den Aufbau von Fettdepots nicht ausgeschöpft wurde. Somit ermöglichten vor allem Fettcharakteristika die Unterscheidung des Ban-Spezialitätenfleisches von in Nordvietnam erzeugtem herkömmlichem Schweinefleisch sowie dessen Positionierung auf dem Fleischmarkt. Darüber hinaus wiesen die Daten der für das Hochpreissegment geschlachteten Ban-Mastschweine (n = 51) darauf hin, dass die derzeitigen Schlachtgewichte aus wirtschaftlicher Sicht suboptimal sind. Eine moderate Anhebung der Schlachtgewichte von derzeit zwischen 12 und 15 kg auf circa 20 kg war unter den gegebenen Produktionsbedingungen machbar, ohne dass die Schlachtkörper- oder Fleischqualität dabei vermindert wurde. Der wirtschaftliche Erfolg der in die Wertschöpfungskette involvierten Akteure könnte daher durch eine auf einem degressiven Preisbildungssystem basierende Vermarktungsmaske verbessert werden und zum Aufbau eines der ersten formalisierten Netzwerke für die Vermarktung von Qualitätsschweinefleisch der Ban-Rasse in Nordvietnam beitragen.

Zusammenfassend deutet diese Arbeit darauf hin, dass der Einsatz nicht-konventioneller und/oder langsam wachsender Schweine- und Hühnertypen an sich keine erhöhte bzw. unterscheidbare Fleischfarbe sowie -qualität bedingt. Es scheint, dass die Inwertsetzung der Fleischprodukte von Monogastriern durch Nischenvermarktung wesentlich von der Kombination der jeweiligen Genotypen mit geeigneten Produktionsmethoden abhängt, wodurch die Bedeutung einer gezielten, passgenauen Wahl des Schlachtalters und der Fütterungsstrategie durch den Produzenten hervorgehoben wird. Diese spezifische Kombination einer lokalen tiergenetischen Ressource mit entsprechenden Produktionsstandards bildet so ein normatives Gefüge, welches die Stabilität eines alternativen Wertschöpfungsnetzwerkes gewährleisten kann. Der Fokus sollte dann auf die nicht minder bedeutsame Entwicklung nachhaltiger Vertriebswege und effektiver Unternehmensstrukturen des betreffenden Produktions- und Vermarktungsnetzwerkes gelegt werden.