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Influence of animal-specific factors on the quality and shelf life of fresh poultry and pork meat

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Meiner Familie

I am among those who think that science has great beauty.

Marie Skłodowska Curie, 1867-1934

physicist and chemist,
first woman to win a Noble Prize,
first and only person to win it twice

Abstract

Influence of animal-specific factors on the quality and shelf life of fresh poultry and pork meat

The objective of this thesis was the assessment of the influence of animal-specific factors on typical quality parameters and shelf life of fresh poultry and pork meat. The trials comprised different study designs to analyze the impact of the animal diet, production system, performance parameters, animal health and terminal sire genotype on the quality and shelf life of the end product. The quality analyses focused on physicochemical parameters, the nutritional value of the filets and biochemical muscle characteristics. The shelf life of the meat was determined by sensory as well as microbial investigations. Additionally, the purchase decision and the occurrence of meat failures such as pale soft and exudative meat as well as White Striping were investigated.

For poultry, the diet had a major impact on the meat quality, but not shelf life, of the breast filets. The meat quality was improved by the supplementation of different concentrations, but not sources, of methionine. However, the supplementation of methionine led to higher filet weights and a higher incidence of White Striping. Severe White Striping had a negative impact on the appearance and purchase decision. The investigation of different broiler production systems revealed an improved nutritional value, muscle characteristics and color of the filets produced in an alternative antibiotics-free husbandry system. The initial microbial quality or shelf life was not different between both production systems. Thus, an extensified and more sustainable production of poultry is possible without negative impacts on meat quality and shelf life.

For pork, an influence of performance parameters on meat quality as well as shelf life was observed. Besides breed and sex, specific quality parameters showed an effect on microbial shelf life. These factors were significantly influenced by the weaning age of the pigs. In contrast, the health status of the animals or antibiotic medication showed no significant effect on shelf life, even though animals with clinical findings displayed a tendency to a shortened microbial shelf life. The optimization of terminal sire line genotype did not result in differences in the shelf life. But a higher susceptibility to stress was observed for one investigation group leading to lower pH-values, a higher drip loss and a higher incidence of PSE meat. Thus, optimization within the Piétrain breed still bears the risk of producing animals more susceptible to stress due to genetic characteristics and with implicit consequences for meat quality.

Zusammenfassung

Einfluss tierspezifischer Faktoren auf die Qualität und Haltbarkeit von frischem Geflügel- und Schweinefleisch

Ziel der vorliegenden Arbeit war die Untersuchung des Einflusses tierspezifischer Faktoren auf die Qualität und Haltbarkeit frischen Geflügel- und Schweinefleisches. Mit verschiedenen Studiendesigns wurde der Einfluss der Fütterung, des Produktionssystems, der Leistungsparameter, der Tiergesundheit und der Endstufengenetik auf das Endprodukt untersucht. Die Qualitätsanalysen konzentrierten sich auf physikochemische Parameter, den Nährwert und biochemische Muskelcharakteristika. Die Haltbarkeit des Fleisches wurde durch sensorische und mikrobiologische Untersuchungen bestimmt. Außerdem wurde die Kaufentscheidung und das Auftreten von Fleischfehlern wie PSE-Fleisch und Weiße Streifen (White Striping) untersucht.

Bei Geflügel hatte das Futter einen großen Einfluss auf die Qualität, jedoch nicht auf die Haltbarkeit der Filets. Die Fleischqualität wurde durch die Fütterung verschiedener Konzentrationen, aber nicht Quellen, von Methionin verbessert. Jedoch führte die Supplementierung von Methionin zu größeren Filetgewichten und einem erhöhten Auftreten von Weißen Streifen, was einen negativen Einfluss auf den Gesamteindruck des Fleisches und die Kaufentscheidung hatte. Die Untersuchung verschiedener Produktionssyteme von Geflügel ergab verbesserte Nährwerte, Muskelcharakteristika und Farbe der Filets aus einem alternativen antibiotika-freien Produktionssystem. Die mikrobiologische Qualität und Haltbarkeit unterschied sich nicht. Eine extensivierte, nachhaltigere Geflügelprodutkion ist demnach ohne negative Auswirkungen auf die Qualität und Haltbarkeit des Fleisches möglich.

Für Schweinefleisch wurde ein Einfluss von Leistungsparametern auf Fleischqualität und Haltbarkeit beobachtet. Neben Rasse und Geschlecht zeigten auch jene Qualitätsparameter einen Einfluss auf die mikrobielle Haltbarkeit, die signifikant vom Absetzalter der Ferkel abhingen. Es wurde kein signifikanter Einfluss des Gesundheitsstatus oder Antibiotikaeinsatzes beobachtet, auch wenn Tiere mit Befund eher zu kürzeren Haltbarkeitszeiten neigten. Die Optimierung der Endstufengenetik führte nicht zu Unterschieden in der Haltbarkeit. Allerdings wurde bei einer Untersuchungsruppe eine höhere Stressanfälligkeit beobachtet, die zu niedrigeren pH-Werten, höherem Tropfsaftverlust und einem vermehrten Auftreten von PSE-Fleisch führte. Daher wurde geschlussfolgert, dass eine Optimierung innerhalb der Piétrain-Linie immer noch das Risiko birgt, durch genetische Charakteristika stressanfälligere Tiere zu produzieren, mit den entsprechenden Konsequenzen für die Fleischqualität.

Contents

1. Introduction

1.1. Quality Parameters and Spoilage Process of Fresh Meat

A high quality of fresh food is crucial for the subsequent processing, stability and salability of the products. With an increasing complexity of production and supply chains, the persistence of food quality and suitability for storage is an important requirement for every actor in the chain. As quality is one major driver for the purchase decision, there is an increasing sensitivity in the food production industry that competing on price alone is not sufficient to satisfy the refining consumer demands (Grunert, 2005). The quality of food is defined as

> *"the sum of value-determining properties of food, which define the degree of utilization for the prescribed purpose." (Hammes, 2004)*

Compared to other fresh products, meat is a product characterized by a particular complexity. The term meat quality comprises a set of different inherent characterics. Meat quality is defined by the compositional quality, the functional quality and the palatability (ElMasry and Sun, 2010; FAO, 2014). The compositional meat quality covers attributes such as the nutritional value, intramuscular fat, marbling, the lean to fat ratio and the meat percentage. The functional quality of meat determines the ability for processing as well as storage and covers, among others, the oxidative stability, water holding capacity, the pH-value and muscle fiber shortening. Finally, meat quality is characterized by the palatability and eating quality which are specified by the appearance, the color, tenderness, flavor or juiciness of the product (ElMasry and Sun, 2010; FAO, 2014). Besides this general definition, the expectations linked to the term meat quality are differing between the supplying sector and the consumers (Grunert et al., 2004). Consumer concerns about ethical issues, animal welfare, health and product safety are rising (Verbeke and Viaene, 2000; Grunert, 2005). Additionally, the environmental impact, production characteristics and origin of the animals are increasingly integrated

in the consumer perception of high quality meat (Font-I-Furnols and Guerrero, 2014; Grunert et al., 2018).

Since fresh meat is no stable product but undergoes different biological, physicochemical and microbial activities, meat quality is a dynamic state which is continuously moving to reduced levels (Taoukis, P. S., Labuza, T. P., Saguy, I. S., 1997). The degradation is variable between different meat types. While "white meat", such as poultry, is very susceptible to deterioration, "red meat" (e.g. pork, beef, lamb) shows a slower loss of quality (Bruckner et al., 2013). Freshness describes the state of highest quality of the product directly after slaughter, without any signs of deterioration. With increasing time, the meat product will degrade in freshness until the product is spoiled.

In general,

> *"spoilage of food involves any change which renders food unacceptable for human consumption and may result from a variety of causes."*
>
> (Forsythe, 1995)

Spoilage can have several causes, such as microbial growth and metabolism, insect harm, physical damage, the activity of intrinsic enzymes as well as chemical processes. For fresh meat, most quality changes during spoilage are initiated by three main mechanisms (Dave and Ghaly, 2011). As a result of microbial activity, the major deteriorative changes which are perceived organoleptically by the consumer are off-odors, the release of metabolites and the formation of slime on the meat surface. Second, lipid oxidation and color changes are biochemical processes related to the spoilage of meat (Huis in't Veld, Jos H.J., 1996). Finally, autolytic enzymatic mechanisms change the appearance of the meat (Dave and Ghaly, 2011).

Fresh meat is distinguished by a high water content, a large amount of nutrients and an optimal pH-value for the growth of microorganisms. The nutritional value may vary between different meat types, but is generally constituted by the main components water (70%), followed by protein (20%), lipids (<10%) and ash (1%)(Lambert et al., 1991; Krämer, 2002; Belitz et al., 2009; Reuter, 2003). Additionally, low molecular weight substances such as glucose, lactic acid, amino acids, nucleotides and urea are main energy resources for metabolic activities (Nychas et al., 1988, 2007). Due to its physicochemical properties and composition, fresh meat is very susceptible to spoilage processes with microorganisms being one major actor during deterioration (Gill, 1983; Forsythe, 1995).

The intermediate and final products of microbial metabolism characterize the spoilage of meat by off-odors, discoloration or slime production (Nychas et al. (2008), see Table 1.1).

Table 1.1.: Characteristics of sensory alteration for different products and the causing organisms

Alteration	Product	Aetiology
Off-Odors		
sweet, fruity	AP meats	*Pseudomonas* spp., *Pseudomonas fragi*
cheesy, rancid	AP meats	Enterobacteriaceae, *B. thermosphacta*, *Lactobacillus* spp.
	MAP meats	*B. thermosphacta*
putrid, sulphury	AP meats	*Pseudomonas* spp.
	VP meats	*Clostridium* spp., *Hafnia* spp.
	Ham	Enterobacteriaceae, *Proteus* spp.
H_2S	Cured meats	Enterobacteriaceae, *Vibrio* spp.
Ammonia	AP meats	*Pseudomonas* spp., *Alcaligenes* spp.
Cabbage odor	Bacon	*Protidencia* sp.
Sour, acid	Ham	LAB, *Alcaligenes* spp., *Micrococcus* spp., *Bacillus* spp.
	VP meats	LAB
Blown pack (H_2, CO_2)	VP meats	*Clostridium* spp., *Alcaligenes* spp., LAB
Discoloration		
H_2O_2 greening	Meats	*Weissella* spp., *Leuconostoc* spp., *Enterococcus* spp., *Lactobacillus* spp.
H_2S greening	VP meats	*Shewanella* spp.
Blue color	Fresh beef	*P. syncyanea*
red spots	Fresh beef	*Serratia marcescens*
Slime production		
Slime	Meats	*Pseudomonas* spp., *Lactobacillus* spp., *Enterococcus* spp.
	Fresh beef	*Acinetobacter* spp., *B. thermosphacta*, *Leuconostoc* spp.
Surface slime	Sausages	*Bacillus* spp., *Lactobacillus* spp., *Leuconostoc* spp.
	Dried meats	*Micrococcus* spp.
Ropy slime	VP cured meats	*Lactobacillus* spp., *Leuconostoc* spp.
Greenish slime	Meats	*Weissella viridescens*

LAB: Lactic acid bacteria, **AP:** aerobical packaged, **MAP:** modified atmosphere packaged, **VP:** vacuum packaged, Based on Dainty and Mackey (1992); Russell et al. (1995); Borch et al. (1996); Nychas et al. (2008); Erkmen and Bozoglu (2016); Iulietto et al. (2016)

The shelf life of meat or meat products is described as the time of storage until the product is spoiled. The point of spoilage is defined as

> *"a certain maximum acceptable bacterial level, or an unacceptable off-odor/off-flavor or appearance. The shelf-life depends on the numbers and types of microorganisms, mainly bacteria, initially present and their subsequent growth."*
>
> (Borch et al., 1996)

During slaughter and processing, fresh meat is contaminated with microorganisms emerging from animal microbiota as well as microorganisms of human or environmental origin. The bacteria are transferred to the product via contaminated machines, surfaces and the aerosols in the slaughterhouse (Mossel, 1971; Bolder, 1998; Rouger et al., 2017; Lues

et al., 2007). Furthermore, the diversity and extent of microbial contamination is also dependent from animal health and husbandry characteristics. The microflora colonizing meat covers a variety of species, connected to the predominant microflora in slaughter and processing facilities (Lues et al., 2007; Rouger et al., 2017). The initial bacterial flora on meat comprises for example *Pseudomonas* spp., lactic acid bacteria, coryneform bacteria, *Bacillus* spp., *Flavobacterium* spp. and *Brochothrix* spp. (Blickstad and Molin, 1983; Borch et al., 1996; Nychas et al., 2007; Rouger et al., 2017). Besides, the presence of pathogenic bacteria such as *Salmonella* spp., *Listeria* ssp., *Campylobacter* spp., *Escherichia coli* or *Staphylococcus aureus* can lead to safety issues in the meat supply chain (Mead, 2004; Nørrung and Buncic, 2008; Rouger et al., 2017). After the initial colonization of the meat, only a small fraction of microorganisms will multiply (Huis in't Veld, Jos H.J., 1996; Nychas et al., 2008). As little as 10% of the initial microflora is able to grow at refrigeration temperatures (Borch et al., 1996). Thus, especially psychrophilic bacteria succeed to compete against others and lead to the deterioration of the final product. These organisms form the microbial 'spoilage association' and are determined by a set of different parameters (Mossel, 1971; Gram et al., 2002). For the growth potential of specific microorganisms on the product, Mossel (1971) defined the *intrinsic*, *extrinsic* and *processing factors* as major impact factors. Additionally, the *implicit factors* combine all factors caused by the development of microorganisms, their interaction, competition, symbiosis as well as the effect of their metabolites. On a particular product, the specific spoilage organism (SSO) is the microorganism which grows dominant and provokes the changes leading to sensory rejection (Gram et al., 2002). By knowing a few physical and chemical properties of the food, the prediction of the SSO and their growth is possible (Gram et al., 2002). For this purpose, predictive microbiology is a powerful tool to calculate the spoilage process and the remaining shelf life of the product at every step in the chain (McDonald and Sun, 1999; Raab et al., 2008). Due to the fast generation time and metabolic characteristics, *Pseudomonas* spp. is the SSO for unprocessed, aerobically packaged meat products (Dainty and Mackey, 1992; Bruckner et al., 2012). Apart from the velocity of deterioration, the spoilage processes of fresh poultry and pork meat are very similar and can be calculated by using the same mathematical models (Bruckner et al., 2012, 2013). This is of particular interest, since these are the meat markets showing the highest growth on a global scale (Henchion et al., 2014). In Europe, the annual meat consumption is 76.5 kg/capita, with pork (34.2 kg/capita) and poultry (21.9 kg/capita) representing the major markets (Font-I-Furnols and Guerrero, 2014). A low price of the product is still an important driver of consumer

choices, but quality and sustainability are gaining in importance (Verbeke et al., 2010; Henchion et al., 2014). On top, a high storage stability and long shelf life is required in increasingly complex supply chains and especially demanded by retailers. A long shelf life of the product offers the opportunity to reduce food waste and enhance the sustainability of meat supply chains. As a result, the meat industry forces an improvement of meat quality and shelf life while maintaining a high efficient production with fast animal turnover rates.

1.2. Influence Factors on Meat Quality and Shelf Life

The factors influencing meat quality and shelf life can be subdivided into four major categories. These factors comprise the complete value added chain, from the animal production to the consumption by the consumer. Each of the four categories can be assigned to one essential element in the production or storage process, including also the properties of the product itself. The influence factors on meat quality and shelf life are subdivided into the *animal-specific factors*, *product specific factors*, *process specific factors* and *environmental factors* (Figure 1.1).

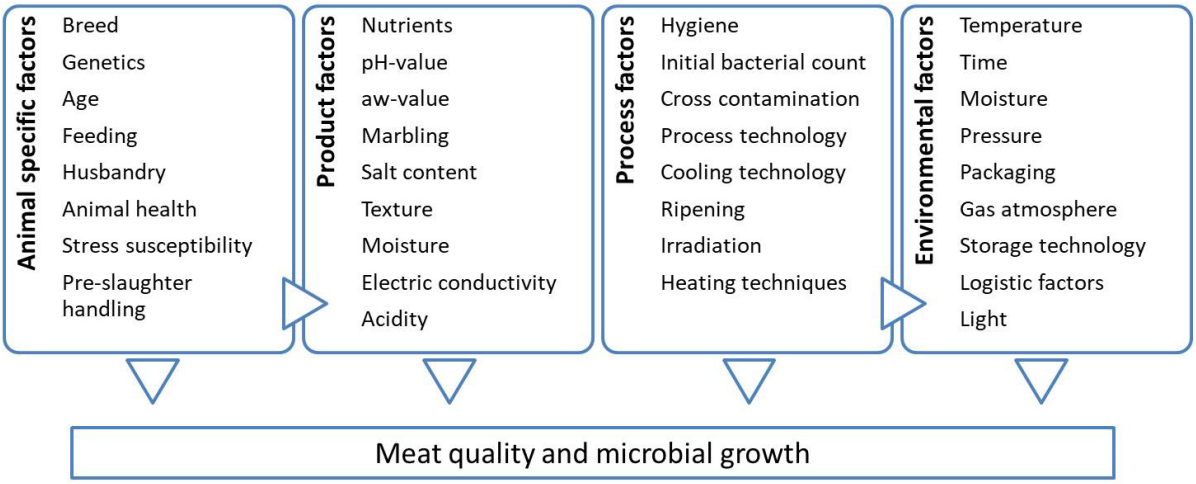
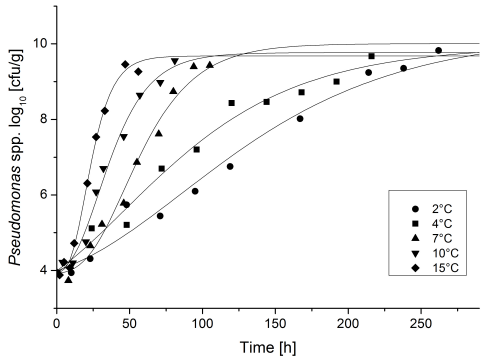


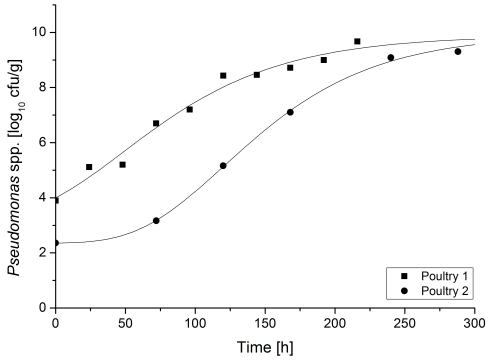
Figure 1.1.: Influence factors on the quality and shelf life of meat. Modified after Kreyenschmidt and Ibald (2012)

For the length of shelf life, the *environmental factors* are of major importance. They are determined by a set of different drivers referring mostly to the storage conditions of

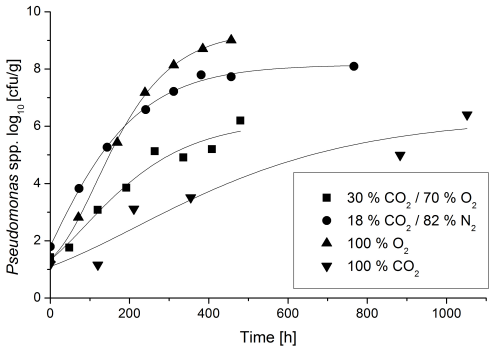
the meat. Intensive research efforts focused on the impact of environmental factors on fresh meat in order to control the cold chain and prolong the shelf life with advanced packaging technologies. The temperature is supposed to have the highest influence on the stability of meat products (Figure 1.2a). Due to its ability to accelerate microbial growth and metabolism as well as biochemical and physical processes, temperature has a crucial impact on the quality, safety and shelf life of meat (Mossel, 1971; Huis in't Veld, Jos H.J., 1996; Nychas et al., 2008). Although the initial contamination of meat covers mesophilic and cold-tolerant species, only the latter, especially the psychrotrophic and psychrophilic bacteria, are found in the spoilage flora of chilled products (Gill and Newton, 1978; Borch et al., 1996). A further selection of the proliferating bacteria will result from the gaseous atmosphere (Mossel, 1971; Gill, 1983). In aerobically packaged meat products, *Pseudomonas* spp. rapidly grows dominant during the competition with other spoilage bacteria (Dainty and Mackey, 1992; Borch et al., 1996; Labadie, 1999). The development of vacuum and *modified atmosphere packaging (MAP)* has derived advantage from the significant influence of the gaseous atmosphere on microorganisms (see Figure 1.2c, Borch et al. (1996); McMillin (2017)). The atmosphere of the packaging significantly affects the microbial composition, competition as well as the velocity of growth. For MAP, different levels of oxygen, carbon dioxide, nitrogen and inert gases affect the spoilage process leading to a shift of the SSO (Borch et al., 1996; Herbert et al., 2015). For fresh poultry stored at 4°C, the shelf life can be prolonged from 100 h (aerob) to 212 h by using a 70%O_2-packaging (Herbert, 2014). Moreover, the presence of particular gases considerably influences meat quality and shelf life, for example with carbon dioxide by reducing the pH of meat and high oxygen levels by saving the fresh red color of meat (Mossel, 1971; Dainty and Mackey, 1992; McMillin, 2008). The absence of oxygen in vacuum packages in combination with microbial activity and a continued respiratory activity of the meat tissue significantly reduces the oxygen content while the tension of carbon dioxide increases (Dainty and Mackey, 1992). This affects, in dependence of time and pH, the predominant microorganisms as well as spoilage characteristics (Gill, 1983). The highest prolongation of shelf life can be achieved by a specifically adjusted atmosphere with respect to the meat characteristics as well as storage conditions (McMillin, 2008, 2017). Additionally, innovations in the field of active and intelligent packaging result in further prolongations of the shelf life or optimization of product handling along the meat supply chain (Kerry et al., 2006; McMillin, 2017). Beside temperature and packaging, the factors pressure, moisture, light and also the storage technology influence the quality and shelf life of meat (Huis in't Veld, Jos H.J., 1996).



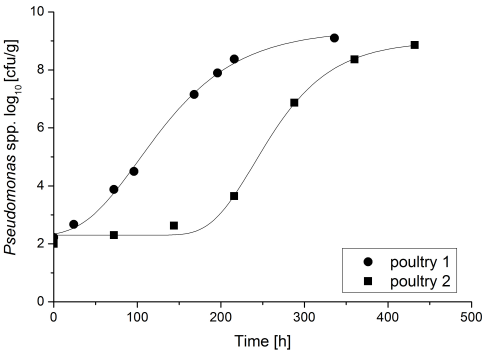
(a) Influence factor: Temperature



(b) Influence factor: Initial bacterial load



(c) Influence factor: Atmosphere



(d) Influence factor: Different product origin and cooling technology, identic storage conditions

Figure 1.2.: Influence factors on the quality and shelf life of fresh poultry meat. Modified after Kreyenschmidt and Ibald (2012)

The *process specific factors* include influence factors within the slaughter and processing facilities. The education of employees, industrial hygiene, equipment and the cleaning routines significantly affect the initial contamination of the product (Mossel, 1971; Bolder, 1998; Rouger et al., 2017). The level of carcass contamination is directly associated to the level of meat contamination, at which a dissemination of microorganisms over the product takes place during different processing steps (Coates et al., 1995). Optimizing hygienic conditions can lead to a significant reduction of microbial contamination, resulting in a prolongation of the shelf life of fresh poultry filets by two days (Figure 1.2b, Bruckner et al. (2013)). Furthermore, cooling technologies are critical for meat hygiene, safety and decelerating microbial growth (Zhou et al., 2010). The rate

of chilling directly after slaughter, evisceration and processing significantly effects the muscle structure, pH decline and protein denaturation of the meat (Dave and Ghaly, 2011). Thus, products processed with different cooling technologies can show varying microbial spoilage processes (Figure 1.2c). Several technological enhancements and food processing treatments have been developed for the preservation of food. For fresh meat, ionizing radiation has the potential to reduce the initial microbial population (Dempster, 1985). Even though *radiation preservation* is proved to increase storage stability of fresh food and a lot of research has been done to disprove possible health risks, it is not approved in all countries (Andrews et al., 1998). Heat steps during food processing are often interconnected for reducing the microbial counts in food (Mossel, 1971). Next to this physical treatment, further processing techniques, such as smoking, lead to a chemical preservation of fresh meat (Mossel, 1971). The applicability of physical and chemical treatments, as well as the supplementation of additives, is limited for fresh, unprocessed meat. Thus, enhanced hygiene management, cooling technology, ripening, process technology and environmental factors build the foundation for high quality products and long shelf life.

The *product specific factors* comprise all intrinsic properties which are typical for fresh meat. The meat composition and the nutritional value influence the storage stability through the availability of nutrients and key substrates, such as glucose (Nychas et al., 1988; Borch et al., 1996). Since meat is a heterogeneous food system with a complex microstructure, its texture and composition also has an impact on microbial growth. The access to nutrients is dependent on mass transport, concentration gradients and diffusion rates in the media (Robins and Wilson, 1994; Wilson et al., 2002). Due to contamination pathways and the access to gaseous compounds, microbial growth originates from the meat surface. Therefore, the structure and moisture of the meat surface significantly affects the colony expansion during the proliferation of microorganisms (Robins and Wilson, 1994). Besides moisture, also the water activity (a_w-value) is of significance for the metabolism, survival and reproduction of microorganisms on meat (Leistner and Rodel, 2012; Esener et al., 1981). The reduction of the water activity by drying, ripening or fermentation prolongs the shelf life. Increasing the salt content in meat is another technique to reduce the a_w-value and decelerate microbial growth. Moreover, adding sodium chloride affects the growth of microorganisms via increasing the osmotic pressure, reacting with alpha-amino groups or iron-containing compounds and blocking sulfhydryl groups, respectively (Dave and Ghaly, 2011). The pH-value is one further product specific factor with major importance for the growth rate of microorganisms

(Gibson et al., 1988; Borch et al., 1996; Dainty and Mackey, 1992). After slaughter, the metabolic supply of the muscles collapses leading to an adjustment to anaerobic metabolic pathways. The metabolism of glycogen via pyruvate leads to an accumulation of lactic acid in the cells, which results in a decrease of the meat pH-value in the first 24 h postmortem (Gill and Newton, 1978; Dave and Ghaly, 2011). These metabolic processes during rigor mortis transform the muscle of the animal into meat, a food product suitable for human consumption (Dave and Ghaly, 2011). The final pH-value depends on the part of the carcass, the fat content, pre-slaughter handling of the animal, as well as the cooling technology during processing (Dainty and Mackey, 1992; Borch et al., 1996; Dave and Ghaly, 2011). Based on the glycolytic potential of the muscle, the pH-value is closely related to the color, the water binding capacity and texture of the meat. In dependency of meat type, high pH levels (>6.0 for red meats) result in Dark, Firm and Dry (DFD) meat, which is caused by long-term stress and deficient pre-slaughter handling (Guàrdia et al., 2010). The high pH in DFD meat is related to an elevated water binding capacity, a dark color and reduced shelf life. Consumers often reject DFD meat due to the appearance and bland taste (Newton and Gill, 1981). An ultimate pH lower than normal leads to Pale, Soft and Exudative (PSE) meat, with remarkable consequences for processing and disposal (Barbut et al., 2005). Besides the stocking density, transportation time and stress prior to slaughter, a few genetic markers have been identified, which can determine the susceptibility of the animal for PSE meat (Barbut et al., 2008; Gajana et al., 2013).

The *animal-specific factors* focus on the first steps in the meat production. Even though the effects of genetic selection and adjusted diets are well investigated, a comprehensive view on meat quality and shelf life from farm to fork is often not considered. The choice of breed has a crucial impact on the meat composition, the fat and protein content, and as a result the meat quality, as well as nutritional value (Cameron, 1990). For the last decades, genetic selection focused on a high growth velocity and enhanced meat yields in the commercial production of pork and poultry, but also led to meat failures such as White Striping or PSE meat (Dransfield and Sosnicki, 1999; Barbut et al., 2008; Kuttappan et al., 2012). The glycolytic potential of the muscle at slaughter, and therefore the ultimate pH of the meat, was shown to be highly heritable, which includes the potential of a targeted selection for particular meat quality parameters in combination with a satisfying meat yield (Monin et al., 1987; Le Bihan-Duval et al., 2008). Additionally, particular production lines are very susceptible to pre-slaughter stress, which leads to a rapid initial pH decline and has direct implications for the sub-

sequent technological processing capabilities and storage stability of the meat (Debut et al., 2003; Ferguson and Warner, 2008; Schwörer et al., 1980). Genetic analyses have revealed some of the genes causing the characteristic traits of poultry and pork breeds. For pork, the halothene gene has been identified as an important driver for feed efficiency, carcass yield, meat quality, as well as the stress resistance of the animals (Leach et al., 1996; Rosenvold and Andersen, 2003a). Next to pre-slaughter stress, the diet has a noticeable impact on the color and color stability of meat (Rosenvold and Andersen, 2003b). Also the leanness, carcass characteristics and fat composition are influenced by the nutrition (Pettigrew and Esnaola, 2001). Particular feeding strategies can be used to manipulate the muscle protein turnover, which is closely related to meat tenderness. Second, the glycolytic potential of the muscle can be regulated by the diet. The glycogen content is a measure for the muscle energy levels and determines the pH decline postmortem, the water holding capacity, as well as the sensory properties of the meat (Andersen et al., 2005; Rosenvold and Andersen, 2003a). For the short-term regulation of the ultimate pH, advanced feeding strategies are applied before slaughter (Guardia et al., 2014). Furthermore, the supplementation of high levels of magnesium shortly before slaughter can reduce the occurrence of PSE meat during pork production (Pettigrew and Esnaola, 2001). The addition of essential amino acids in broiler diet, such as methionine or lysine, enhances performance parameters, meat yield and final body weight. Besides, the diet also regulates the final pH, drip loss and color of poultry meat (Berri et al., 2008; Wallis, 1999; Wen et al., 2016). The adjustment of the diet is often accompanied with a changed growth velocity and performance of the animals, which is also considered during the development of alternative husbandry systems. Organic production systems with outdoor access, enhanced roaming, adjusted nutrition and the targeted choice of slow growing races result in significant differences in certain meat quality parameters, compared to the conventional industrial meat production (Fanatico et al., 2007; Castellini et al., 2002; Fanatico et al., 2009). The opportunity of gaining outdoor access as well as the application of fast or slow growing races has a considerable influence on the palatability, more precisely the color or tenderness, and also on the nutritional value by affecting the fat or protein content of the meat (Mikulski et al., 2011). Additionally, the complex impact of animal welfare on meat quality variation is receiving more attention from producers and consumers (Bessei, 2006; de Jonge and van Trijp, Hans C. M., 2013; Verbeke and Viaene, 2000). Next to other authors, Klauke et al. (2013) and Rocha et al. (2016) showed the influence of animal health and welfare on performance, carcass composition and meat quality traits, especially in the first steps

of production.

The quality and shelf life of fresh meat is crucial for the disposal within a certain period in increasingly complex supply chains. As the meat market reached the saturation point in western countries, the requirements of consumers increased regarding high quality and sustainable products (Verbeke et al., 2010; Henchion et al., 2014). Moreover, ethical concerns on animal welfare and health are important drivers for the purchase decision of the consumer (Borell and Sørensen, 2004; Grunert, 2005; Magdelaine et al., 2008; Leinonen and Kyriazakis, 2016; Troy et al., 2016; Castellini et al., 2012). The challenge the meat industry faces today, to address the aforementioned issues, is how to efficiently produce affordable products with high quality standards under the consideration of sustainability, animal welfare and health at the same time. The efforts of the production sector to satisfy these conflicting demands led to the establishment of different production systems and continuing improvements (Verbeke and Viaene, 2000; Trienekens et al., 2009). During the optimization of animal production, meat quality investigations mostly focus on carcass characteristics, quality traits and palatability directly after slaughter. Even though the impact of animal-specific factors on pH, water holding capacity and especially meat composition is well documented, comprehensive approaches from farm to fork are lacking. The storage capability of the end product is often not considered, even though typical meat quality parameters are known to have a striking impact on microbial growth and shelf life. The influence of animal-specific factors on meat quality is unquestionable, but how they affect the stability of quality or the shelf life of the product has not been investigated yet.

1.3. Research Questions and Outline of the Thesis

The main objective of this thesis is the investigation of animal-specific parameters as influence factors on the quality and shelf life of fresh poultry and pork meat. For this purpose, the following research questions are formulated:

- How are meat quality and shelf life influenced by the supplementation of a growth promoting amino acid in the diet?
- Do different industrial production systems focusing on optimized breed, diet, animal welfare and handling of antibiotic medication have an effect on the quality and shelf life of fresh meat?
- How is the rearing, growth performance, animal health and welfare related to the quality and shelf life of the product?
- Is quality optimization via terminal sire line selection possible without any impacts on animal health, meat quality and shelf life?
- How does the optimization of production systems influence the consumer perception of quality and purchase decision?

In the first part of the thesis (*chapter 2* and *chapter3*), the influence of the diet on the quality and spoilage of fresh poultry meat was investigated. The effect of different doses and sources of methionine in the diet was analyzed for six treatment groups and one control within fully controlled experiments in a laboratory environment (*chapter2*). Typical meat quality parameters, such as pH-value and drip loss, were assessed during an aerobically storage of the poultry filets at 4°C. Moreover, microbial investigations, focusing on total viable count (TVC) and *Pseudomonas spp.*, were conducted at the beginning and end of storage. Sensory characteristics were investigated to assess the shelf life of the poultry filets and examine the purchase decision of the consumer. The trial was repeated with adjusted methionine concentrations under commercial fattening and slaughter conditions (*chapter 3*). Meat failures such as White Striping were assessed. As an effect of methionine supplementation, the influence of meat color and White Striping on the consumer acceptance was investigated, and mapped by a three-dimensional surface model.
Chapter 4 of the thesis focuses on the comparison of two husbandry systems in industrial poultry production. The investigation comprised one conventional and one alternative production line. The alternative production line included a slow-growing poultry race,

a corn-based diet, more place for roaming and the strict absence of antibiotics. The nutritional content of the filets was determined and meat quality parameters were assessed during the aerobical storage of the filets at 4°C. In addition, microbial as well as sensory spoilage was investigated and shelf life of the poultry filets was calculated.
The next part of the thesis (*chapter 5*) deals with the influence of animal health, animal welfare and husbandry of pigs on the meat quality and shelf life of fresh pork meat. Investigations of meat quality, microbial and sensory parameters were conducted to determine the shelf life of the filets. Performance parameters, as well as rearing and fattening characteristics of the pigs, were assessed to calculate their influence on the quality and shelf life of fresh pork meat. Moreover, animal health parameters were tested for their influence on the storage stability of the product.
In the last chapter (*chapter 6*), terminal sire line selection was examined as optimization opportunity for pork meat quality. Two boar lines of German Piétrain were investigated within an industrial production system and compared to the conventional production line. A nutritional analyses was performed as well as an examination of the storage stability. Pork samples of the *Musculus longissimus dorsi* were stored aerobically at 7°C. The investigation of the samples comprised meat quality parameters, the assessment of microbial parameters and the sensory index to determine the shelf life of the product. The data gained from the laboratory was combined with health and Auto-FOM (Fat-O-Meter) data of the slaughterhouse.

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2. Effect of Methionine Supplementation in Chicken Feed on the Quality and Shelf Life of Fresh Poultry Meat

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2.1. Abstract

The aim of this study was to investigate the influence of different methionine sources and concentrations on the quality and spoilage process of broiler meat. The trial was comprised of 7 treatment groups: one basal group (suboptimal in Methionine+Cysteine; i.e., 0.89, 0.74, 0.69% in DM SID Met+Cys in starter, grower, and finisher diets, respectively) and 3 doses (0.10, 0.25, and 0.40%) of either DL-Methionine (DLM) or DL-2-hydroxy-4-methylthio butanoic acid (DL-HMTBA) on an equimolar basis of the DLM-supplemented groups. The broilers were fed the diets for 35 d, then slaughtered and processed. The filets were aerobically packed and stored under temperature-controlled conditions at 4°C. Meat quality investigations were comprised of microbial investigations (total viable count and *Pseudomonas* spp.), pH and drip loss measurements of the filets. The shelf life of the meat samples was determined based on sensory parameters. After slaughtering, all supplemented meat samples showed a high quality, whereby no differences between the 2 methionine sources could be detected for the microbial load, pH, and drip loss. In comparison to the control group, the supplemented samples showed a higher sensory quality, characterized by a fresh smell and fresh red color. Methionine supplementation had a significant influence on meat quality parameters during storage. The microbial load, pH and drip loss of the chicken filets were positively correlated to the methionine concentration. Additionally, the microbial load at the end of storage was positively correlated to pH and drip loss values. Nevertheless, the microbial parameters were in a normal range and the positive correlation to methionine concentration did not affect the sensory shelf life. The mean sensory shelf life of the broiler filets varied between 7 to 9 d. During storage, no difference in the development of sensory parameters was observed between the supplemented groups, while the spoilage process of the basal group occurred slightly faster. In conclusion, methionine concentration, but not methionine source, effected meat quality parameters in breast muscles of broilers.

2.2. Introduction

Methionine (Met) is an essential amino acid which is commonly used as a supplement in broiler diets. As a sulfur-containing amino acid, the availability of Met is crucial for several metabolic pathways, i.e., synthesis of proteins, transsulfuration, and methylation of DNA (Bunchasak, 2009; Jankowski et al., 2014). Besides acting as a base for carnitine and glutathione synthesis, Met has a positive effect on the expression of stress-related

genes and thus helps to protect cells against oxidative stress (Fang et al., 2002; Li et al., 2007; Luo and Levine, 2009; Del Vesco et al., 2013). Additionally, the immune response of Met supplemented animals is enhanced due to an improved proliferation of immune cells and antibodies (Tsiagbe et al., 1987; Rubin et al., 2007; Maroufyan, 2010). Met is supplemented during the fattening of broilers, resulting in a better performance of animals and an increased growth of breast and leg muscles (Daenner and Bessei, 2003; Motl et al., 2005; Liu et al., 2006). It is evident that feeding broilers with increasing concentrations of Met leads to a decrease in abdominal fat and an increase in growth rate, breast muscle yield, and leg muscle yield (Wallis, 1999; Mandal et al., 2004; Liu et al., 2006).

Additionally, Met uptake and utilization may vary in relation to the Met source used (Richards et al., 2005; Sangali et al., 2015). Several studies aimed to compare DL-methionine (DLM) and DL-2-hydroxy-4-methylthio butanoic acid (DL-HMTBA) concerning metabolic pathways and efficiency of the supplementation (Daenner and Bessei, 2003; Sauer et al., 2008). DL-HMTBA differs from DLM in its molecular structure, absorption, and transformation to Met (Richards et al., 2005). Moreover, there is a difference in growth of animals when comparing the two Met sources (Lemme et al., 2002; Daenner and Bessei, 2003; Motl et al., 2005; Liu et al., 2006; Zou et al., 2015), showing their bio-efficacy is different, as several meta-analyses indicate (Meirelles et al., 2003; Vazquez-Anon et al., 2006; Sauer et al., 2008; Vedenov and Pesti, 2010; Sangali et al., 2015). Although extensive research on the effect of Met supplementation on growth performance and meat yield has been conducted, the effect of Met on meat quality is scarcely investigated. Although a positive influence of Met supplementation on meat color and nutritional composition was reported (Liu et al., 2006; Zhan et al., 2006; Conde-Aguilera et al., 2013), the further improvement in animal performance and its effect on meat quality needs to be elucidated. More generally, a high growth rate can lead to negative morphological deviations of muscle structure and fiber composition (Dransfield and Sosnicki, 1999; Woelfel et al., 2002). Additionally, meat failures such as (pale, soft and exudative meat), White Striping (WS) and wooden breast effect could be related to fast growth rates and high weights of broilers (Dransfield and Sosnicki, 1999; Kuttappan et al., 2012; Petracci and Cavani, 2012; Mudalal et al., 2015). The nutritional value, such as the protein and fat content, may also be influenced by the growth rate (Fanatico et al., 2007). However, these effects were not specifically related to the supplementation of Met. Aksu et al. (2007) and Liu et al. (2006) reported a relationship between Met supplementation and the water content as well as an increasing

meat pH-value. Additionally, the supplementation of DL-HMTBA can decrease lipid oxidation during storage (Berri et al., 2012), but there is a lack of information on how this is going to affect the microbial spoilage process and the shelf life of the products. Intrinsic parameters such as water activity, pH, nutrients, and structure of the meat are strongly related to the spoilage process of the meat (Gill, 1983; Huis in't Veld, Jos H.J., 1996). Raw poultry meat is especially sensitive to microbial spoilage due to its physicochemical properties (Bruckner et al., 2012a). Thus, poultry meat may react noticeably to physicochemical changes induced by dietary modifications. Even though an influence of Met supplementation on the microflora of poultry breast meat and drumsticks was shown by Aksu et al. (2007), there is a lack of studies investigating the relationship between feed composition and the spoilage process of the meat. Studies on the effect of feed composition on quality loss and shelf life are also rare. Most of the studies only focus on typical quality parameters after the slaughtering process, while the effect on freshness parameters during the storage process is not considered. To our knowledge, there are also no studies available which take into account the effect of dietary Met supplementation on the development of typical sensory and microbial parameters during storage.

Thus, the overall objective of this study was to investigate the effect of different concentrations of DL-HMTBA and DLM or a methionine + cysteine (Met+Cys) suboptimal diet on the meat quality parameters and shelf life of breast filets and thighs of broilers after a 35-d growing period.

2.3. Material and Methods

2.3.1. Study Design

One hundred five male broilers (Cobb 500) were fed with different concentrations and sources of Met arranged in 7 treatment groups. A basal diet (suboptimal in Met+Cys; i.e., 0.89, 0.74, 0.69% in DM SID Met+Cys in starter, grower and finisher diets, respectively) was prepared and 3 doses (0.10, 0.25, and 0.40%) of either DLM or DL-HMTBA on an equimolar basis were added to the basal diet (Table 2.1).

Table 2.1.: Ingredient and nutrient composition of the basal diets fed during the starter (d 1-10), grower (d 11-21) and finisher (d 22-35) period.[1]

	Starter, %	**Grower, %**	**Finisher, %**
Ingredient, % fresh matter			
Wheat	31.4	23.4	24.1
Soybean meal	31.9	23.7	25.9
Corn	20	20	20
Peas	-	17	17
Corn gluten meal	7.69	5.59	1.19
Soybean oil	4.12	5.35	6.71
Dicalcium phosphate	1.91	-	-
Monocalcium phosphate	-	1.64	1.66
Limestone ($CaCO_3$)	0.9	1.53	1.93
Mineral and vitamin premix[2]	1	1	1
Sodium bicarbonate	0.12	0.14	0.15
Salt (NaCl)	0.27	0.27	0.27
Biolys® (L-Lysine)	0.61	0.35	0.1
ThreAMINO® (L-Threonine)	0.08	0.06	0.03
ValAMINO (L-Valine)	0.03	0.03	
Nutrient composition, % DM			
DM	89.3	87.5	86.2
Crude ash	7.02	6.69	7.71
Crude fiber	3.63	4.47	4.66
Crude fat	6.81	8.99	9.65
Crude protein	29.9	24.3	23.3
Methionine[3]	0.43	0.35	0.32
Methionine + Cysteine	0.89	0.74	0.69

[1]Starter and Grower diets contained an anticoccidiostatic drug (Maxiban, 0.6g/kg; on top).
[2]The premix supplied per kg diet: Ca, 3 g, Cl, 0.1 g, vitamin A, 12,000 IU, vitamin D3, 4,000 IU, vitamin E, 50 mg, vitamin K3, 3.33 mg, biotin, 250 μg, folic acid, 1.67 mg, vitamin B1, 3.33 mg, vitamin B2, 8 mg, Vitamin B6, 4.17 mg, vitamin B12, 25 μg, nicotinamide, 69.1 mg, calcium pantothenate, 20 mg, choline chloride, 400 mg, Fe, 50 mg, Cu, 15 mg, Mn, 100 mg, Zn, 70 mg, J, 1.56 mg, Se, 0.25 mg.
[3]standardized ileal digestible methionine.

The animals were slaughtered in house (Gießen, Germany) after a 35-d-feeding period in a 3-phase feeding (Starter, 0–10d; Grower, 11–21d; Finisher, 22–35d). Feeding and slaughtering of the animals was conducted by University of Gießen. Dissection, transportation, and all further investigations were conducted by the University of Bonn. Broilers were stunned using a wooden club and exsanguinated with an incision to the carotid artery and placement in a bleeder funnel. De-feathering was performed by submersion in a 65°C water bath and use of an automatic plucker. Immediately after slaughtering and de-feathering, and without cooling, the broilers were processed. The filets were taken under sterile conditions and the skin was removed. Then, the filets were chilled until transportation. The filets were transported to the laboratory under temperature-controlled conditions in insulated boxes with cooling packs. The samples were placed individually in polypropylene trays with lids and were stored at 4°C in low-temperature, high-precision incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan). The storage temperature was monitored by data loggers (ESCORT JUNIOR Internal Temperature Data Logger, Escort, New Zealand), with measurements taken every 5 minutes. The first investigation started approximately 24 h after slaughter, and 35 left filets were analyzed for the following parameters: Microbiological parameters (total viable count, *Pseudomonas* spp.), physicochemical parameters (meat pH-value, drip loss), sensory investigation (color, odor, texture). Following one sensory investigation after 144 h, the next full investigation block was after 192 h for 35 right filets with a repetition of the measurements of 24 h. The trial was conducted in 3 replications with 35 animals each (105 broilers in total).

2.3.2. Physicochemical Parameters

Meat pH-value

The surface pH of the filets was measured 24 h and 192 h after slaughter on the dorsal surface of the filets, using a portable surface pH-meter (pH 8011, Peter Bock Umwelttechnik, Gersfeld, Germany). Three measurements were performed for each meat sample by placing the electrode onto the meat surface and an average pH-value was calculated.

Measurement of the Drip Loss

Drip loss was measured in breast filets, beginning 24 h and 192 h after slaughtering. The breast filets were hung on hooks through their thickest part. To reduce evaporation, the filets were packed in plastic bags and sealed with rubber bands, with the hooks

extending out of the bags. The filets in bags were then hung from a grate for 24 h in a 4°C incubator (bag method after Honikel (1998)). The samples were weighed before and after hanging. Drip loss was calculated as the difference in weight, corrected for mass and expressed as a percent (equation 2.1).

$$D_L = \frac{m_1 - m_2}{m_1} \cdot 100\% \tag{2.1}$$

where D_L is drip loss [%], m_1 is mass before hanging, and m_2 is mass after hanging.

2.3.3. Microbiological Analyses

The microbial analysis was conducted 24 h and 192 h after slaughter to determine the total viable count (TVC) as well as the *Pseudomonas* spp. (Pse) count, the specific spoilage organism for fresh, aerobically packed poultry. For the microbiological analysis, 25 g of surface meat tissue with a size of 4 × 7 × 0.5 cm, were aseptically taken using a sterile scalpel. The sample was transferred to a filtered, sterile stomacher bag and filled with 225 ml saline peptone diluent (0.85% NaCl with 0.1% peptone Saline-Tablets, Oxoid BR0053G, Cambridge, United Kingdom). Samples were mixed with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60 s. Ten-fold dilutions of the homogenate were prepared in saline peptone diluents. The TVC was determined by pour plate technique on Plate Count Agar (PCA, Merck, Darmstadt, Germany). The plates were incubated at 30°C for 72 h. *Pseudomonas* spp. (Pse) were determined by spread plate technique on *Pseudomonas* Agar with Cetrimide-Fucidin-Cephalosporine selective supplement (CFC, Oxoid, Cambridge, United Kingdom). Plates were incubated at 25°C for 48 h.

2.3.4. Sensory Investigation

A trained sensory panel evaluated the samples 24 h, 144 h, and 192 h after slaughter according to a graded 3-point scoring system, with 3 meaning fresh and high quality and 1 meaning unacceptable. The characteristics color, odor, and texture were assessed for each sample. The Sensory Index (SI) was calculated as a weighted average with the following equation 2.2:

$$SI = \frac{2 \cdot O + 2 \cdot C + 2 \cdot T}{5} \tag{2.2}$$

where SI is Sensory Index, O is odor, C is color and T is texture.

According to the scheme, the product is spoiled when the SI reaches the level of 1.8. The SI was plotted as a function of time and fitted to a linear model. Thus, the shelf life of each sample was calculated by equation 2.3 as follows (Kreyenschmidt, 2003):

$$SL = \frac{1.8 - a}{b} \tag{2.3}$$

where SL is shelf life, a is the intercept of the linear model, and b is the slope of the linear model.

Samples exhibiting an atypical spoilage process (no degradation in color or texture) were not included in the statistical analyses of sensory characteristics. Samples with a shelf life lower than 100 h and higher than 300 h were judged as outliers and excluded from the data set, leading to a sample size of 93.

2.3.5. Data Analysis and Statistics

The data were tested for normal distribution and homoscedasticity. Since the data did not meet the conditions for parametric statistical tests, non-parametric methods were used. For illustrating data distribution, boxplots were used displaying median as well as first and third quartiles of data. Differences between groups were tested with the Mann-Whitney-U-Test. Correlations were tested with Spearman's Rank-Order Correlation Test and the correlation coefficient k was computed (with $k < 0.4$ meaning a low correlation, $0.4 < k < 0.6$ meaning a medium correlation, and $k > 0.6$ meaning a high correlation). Test results are marked with * ($P < 0.05$) for significant and ** ($P < 0.001$) for highly significant differences or correlations.

Data analysis was conducted with SPSS Statistics 22 (IBM Corp. 1989, 2013, New York, NY) and OriginPro 8 G (OriginLab Corp., Northampton, MA). Additionally, statistical software R 2.15 (R Development Core Team) was used.

2.4. Results and Discussion

The samples showed a high initial quality upon arrival at the laboratory. Filets of the supplemented groups had a fresh, pink color and fresh smell characteristics, whereas the basal group showed a trend towards lower SI values. However, this effect was not significant and mostly caused by a devaluation of color and odor. Further visual differences between samples were primarily in size (Table 2.2).

Table 2.2.: Meat quality at the beginning (24 h) and at the end of storage (192 h) (n = 105 [15 per group] within a row)

	Basal	DLM 0.10%	DLM 0.25%	DLM 0.40%	DL -HMTBA 0.10%	DL -HMTBA 0.25%	DL -HMTBA 0.40%
Filet weight [g]	134.33 ± 27.9	271.13 ± 25.4	268.67 ± 37.5	272.07 ± 27.1	239.87 ± 36.3	261.60 ± 34.5	293.27 ± 35.6
TVC_{24} [log_{10} cfu/g]	4.04 ± 0.24	3.77 ± 0.56	3.42 ± 0.46	3.82 ± 0.34	3.87 ± 0.36	3.84 ± 0.43	3.71 ± 0.52
TVC_{192} [log_{10} cfu/g]	5.14 ± 1.02	6.04 ± 1.07	6.03 ± 1.06	6.40 ± 1.12	5.76 ± 0.95	6.29 ± 1.17	5.93 ± 1.19
Pse_{24} [log_{10} cfu/g]	1.25 ± 0.55	1.35 ± 0.61	1.29 ± 0.54	1.35 ± 0.54	1.39 ± 0.50	1.27 ± 0.47	1.57 ± 0.58
Pse_{192} [log_{10} cfu/g]	5.82 ± 0.49	6.82 ± 0.97	6.89 ± 0.88	7.23 ± 0.90	6.58 ± 0.68	7.11 ± 0.80	7.13 ± 1.07
pH_{24}	5.76 ± 0.12	6.01 ± 0.11	5.98 ± 0.15	5.95 ± 0.13	5.92 ± 0.09	5.95 ± 0.12	5.99 ± 0.22
pH_{192}	5.76 ± 0.14	5.99 ± 0.11	5.94 ± 0.14	6.01 ± 0.15	5.97 ± 0.12	5.97 ± 0.11	6.00 ± 0.14
Drip $loss_{24}$ [%]	0.74 ± 0.29	0.69 ± 0.18	0.88 ± 0.46	0.80 ± 0.47	0.42 ± 0.16	1.02 ± 0.43	1.21 ± 0.46
Drip $loss_{192}$ [%]	0.63 ± 0.28	0.74 ± 0.28	1.11 ± 0.66	0.88 ± 0.46	0.63 ± 0.29	0.97 ± 0.45	1.37 ± 0.63
SI_{24}	2.76 ± 0.20	2.82 ± 0.13	2.79 ± 0.20	2.85 ± 0.17	2.83 ± 0.15	2.84 ± 0.14	2.82 ± 0.17
SI_{192}	1.72 ± 0.24	1.93 ± 0.19	1.80 ± 0.24	1.81 ± 0.22	1.96 ± 0.17	1.88 ± 0.19	1.85 ± 0.27
Shelf life [h]	176.13 ± 36.12	210.04 ± 40.38	219.13 ± 37.10	195.67 ± 39.43	210.39 ± 43.03	193.94 ± 35.02	197.37 ± 45.62
Shelf life [d]	7	9	9	8	9	8	8

The mean pH-values of all investigated samples (basal as well as supplemented groups) ranged between 5.76 and 6.01 after 24 h (Table 2.2). The typical pH for fresh poultry breast filets 24 h after slaughter ranges between 5.8 and 6.2 (Berri et al., 2001; Garcia et al., 2010; Bruckner et al., 2012a). The pH-values did not differ between DLM and DL-HMTBA ($P > 0.05$, Table 2.3) after 24 h and 192 h, independent of concentration. Moreover, the pH-value did not show a significant change during storage, independent of treatment group. Correlation analysis revealed a low correlation between pH-value and Met concentration after 24 h (k: 0.266, $P < 0.001$) as well as after 192 h (k: 0.322, $P < 0.001$; for an overview of correlation tests, see Table 2.4). In contrast, the basal group deviated significantly from the supplemented groups ($P < 0.001$, Table 2.3) with a mean pH-value of 5.76 ± 0.12 after 24 h and 5.76 ± 0.14 after 192 h. The minimum pH-value measured in the basal group was 5.57, which is normally considered as characteristic for

Table 2.3.: Differences between groups of physicochemical parameters (n=105 [15 per group]), bold values display significant differences.

	Basal	DLM	DL-HMTBA	Basal
Filet weight [g]	**<0.001**	0.586	**<0.001**	
TVC_{24} [log_{10} cfu/g]	**0.002**	0.102	**0.049**	
TVC_{192} [log_{10} cfu/g]	**0.004**	0.499	**0.014**	
PSE_{24} [log_{10} cfu/g]	0.519	0.333	0.182	
PSE_{192} [log_{10} cfu/g]	**<0.001**	0.728	**<0.001**	
pH_{24}	**<0.001**	0.311	**<0.001**	
pH_{192}	**<0.001**	0.831	**<0.001**	
Drip $loss_{24}$ [%]	0.754	0.363	0.465	
Drip $loss_{192}$ [%]	**0.040**	0.368	**0.014**	
SI_{24}	0.155	0.809	0.134	
SI_{192}	**0.041**	0.274	**0.012**	
Shelf life [d]	**0.014**	0.369	**0.004**	

PSE-like (pale, soft, exudative) meat (Woelfel et al., 2002). The positive effect of Met supplementation in glutathione synthesis and expression of oxidative stress-related genes (Wang et al., 2009; Del Vesco et al., 2015) is possibly lacking in the control group. Thus, the deficiency of Met in the feeding probably led to metabolic stress and to the lower pH-value of the basal group. Despite the low pH after 24 h, no other characteristics

Table 2.4.: Correlation coefficients for meat quality parameters of filets (n=105 [15 per group]). Only significant correlations are stated. Bold and italic numbers are significant at the 0.01-level.

	Filet weight	Met. conc.	Shelf life	TVC_{24}	TVC_{192}	PSE_{24}	PSE_{192}	pH_{24}	pH_{192}	Drip $loss_{24}$
Met conc.	***0.565***									
Shelf life	***0.227***									
TVC_{24}	***-0.217***									
TVC_{192}		0.238	***0.524***	***0.266***						
PSE_{24}			0.229	**0.348**	0.241					
PSE_{192}	0.288	**0.452**	**0.448**	**0.326**	**0.708**	**0.344**				
pH_{24}	**0.517**	**0.266**	**0.657**		**0.596**	**0.223**	**0.561**			
pH_{192}	**0.450**	**0.322**	**0.603**		**0.588**	**0.253**	**0.557**	**0.796**		
Drip $loss_{24}$	-0.346	**0.325**			0.220		**0.382**	**0.300**		
Drip $loss_{192}$	**0.459**	**0.392**					**0.261**	0.226		**0.720**

of typical PSE-like failures could be observed. The mean drip loss after 24 h varied between 0.42% and 1.21% for all groups (Table 2.2). These values are in a normal range for fresh poultry (Woelfel et al., 2002; Le Bihan-Duval et al., 2008). At the beginning of storage, no significant differences in the drip loss of breast filets could be detected between the different groups ($P > 0.05$). After 192 h of storage, the drip loss showed an increase in all treatment groups except the control group. A high variation in the drip

loss of breast filets could be observed, particularly at the end of storage. The lower drip loss values of the control group were accompanied by a dry appearance of the surface. Low drip loss values for the control group may be explained by a higher susceptibility to premortal stress in comparison to the Met-supplemented groups. Additionally, there are no statistical significant differences between DLM and DL-HMTBA groups, but differences between the Met-supplemented groups and the basal group were significant at the 0.05-level at the end of storage. However, a linear regression analysis revealed a significant positive correlation between Met concentration and drip loss for the DL-HMTBA treatment groups, but not the DLM group (Figure 2.1).

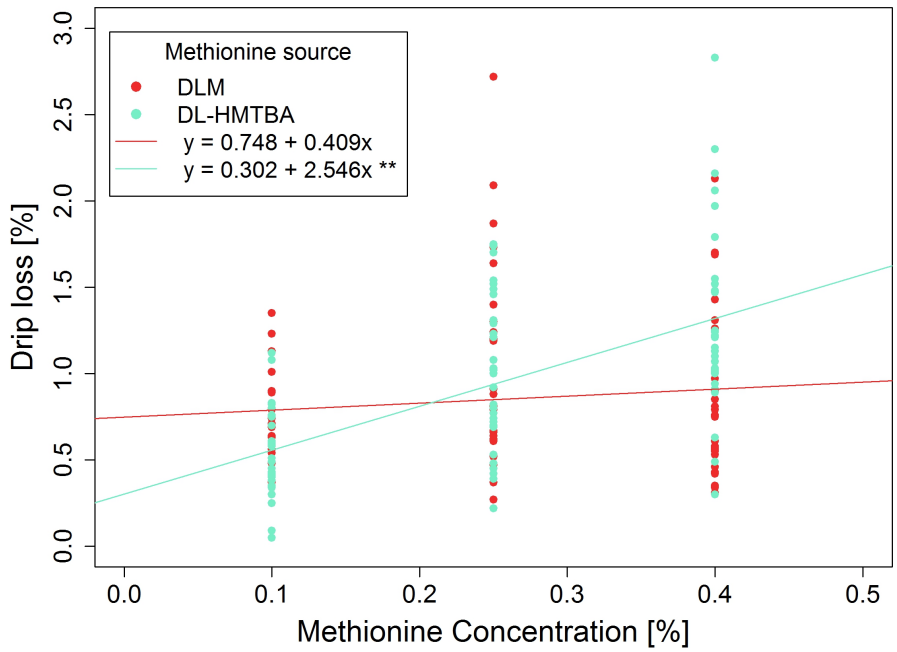

Figure 2.1.: Relationship between drip loss and concentration of both methionine sources with linear modeling and ** for $P < 0.01$

Several studies point to a reduced water holding capacity in fast growing broilers which is supposed to be caused by genetic selection and morphological deviances due to fast muscle growth (Dransfield and Sosnicki, 1999; Mikulski et al., 2011; Petracci and Cavani, 2012). However, the diet of the animals may also have an effect on water holding capacity (Young et al., 2004; Berri et al., 2008; Jiang et al., 2009; Wang et al., 2009). Spearman's Rank Correlation revealed significant correlations between drip loss and filet weight as

well as Met concentration independent of the source (Table 2.4). Thus, an influence of dietary Met on the drip loss, maybe by a faster muscle growth, is possible, even if it has not been reported yet. However, a reduction of drip loss by the addition of Met was reported by Jiang et al. (2009). Further investigations are needed to investigate the influence of Met on the drip loss considering Met concentration and source. The average TVC of all samples 24 h after slaughtering ranged between 3.42 and 4.04 $\log_{10}$ cfu/g (Table 2.2). The initial bacterial count of DLM, DL-HMTBA and the control group were in the same range and comparable to TVC of industrial processed poultry filets (Balamatsia et al., 2006; Bruckner et al., 2012a). The mean initial bacterial count of *Pseudomonas* spp. ranged between 1.25–1.57 $\log_{10}$ cfu/g, which is low in comparison to industrial slaughtering and processing, where *Pseudomonas* spp. dominates the initial flora (Balamatsia et al., 2006; Bruckner et al., 2012a). There was no significant difference for *Pseudomonas* counts between treatment groups at the beginning of storage, meaning that all filets showed a comparable initial contamination by microorganisms (Figure 2.2). After 192 h, mean TVC of the supplemented groups and the control group ranged between 5.14 and 6.40 $\log_{10}$ cfu/g, whereas the supplemented groups showed higher total viable counts than the control group ($P = 0.049$). The mean counts for *Pseudomonas* spp. ranged between 5.82 and 7.23 $\log_{10}$ cfu/g at the end of storage (after 192 h). Similar to the TVC, the count of *Pseudomonas* spp. was higher in the supplemented groups after 192 h ($P < 0.001$) than the counts in the control group. According to Bruckner et al. (2012a), the microbial rejection level for *Pseudomonas* spp. is 7.5 $\log_{10}$ cfu/g. The level is normally reached after 6 to 8 d by industrially processed poultry meat stored aerobically at 4°C (Balamatsia et al., 2006; Bruckner et al., 2012b). In the study, only 15.2% of the DLM and 12.4% of the DL-HMTBA treated groups reached this value after 8 d (192 h). None of the samples of the basal group achieved the microbial rejection level after 192 h (Figure 2.2).

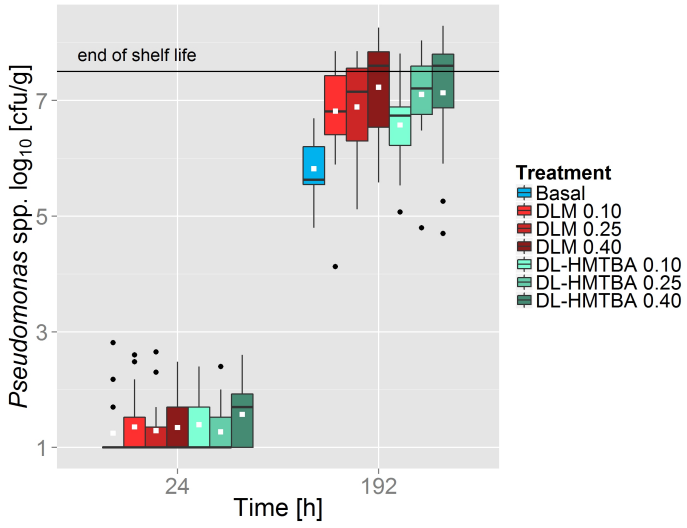

Figure 2.2.: Development of *Pseudomonas* sp. 24 h and 192 h after slaughtering (n = 105 [15 per group]).

Besides the differences in microbial counts at the end of storage, a low to medium correlation between Met concentration and microbial counts could be shown for TVC (k:0.238, $P < 0.05$) as well as for *Pseudomonas* spp. (k: 0.452, $P < 0.001$), which could be explained by a higher drip loss in comparison to the basal group. The differences in the development of microbial counts between control and supplemented groups could be caused by the higher surface moisture, drip loss and pH-value exhibited by the DLM and DL-HMTBA groups in comparison to the basal group. The basal group showed an atypical dry surface, which could be caused by different slaughtering and processing conditions in comparison to commercial slaughtering. Especially the cooling and processing procedure was different in comparison to commercial slaughtering, which may have led to changes in muscle pH and drip loss. The higher drip loss and the higher pH-values provide optimal conditions for the growth of psychrotrophic bacteria, which could lead to an accelerated microbial growth and spoilage (Borch et al., 1996; Huis in't Veld, Jos H.J., 1996; McMeekin and Ross, 1996). The sensory investigations showed a high sensory quality of the meat samples of the supplemented groups regarding the test parameters i.e., odor, color, and texture at the beginning of storage (Table 2.2, Figure 2.3). According to the assessment scheme, the high quality of the meat is reflected by mean values above 2.7 (where 3 = highest quality). There were no differences between treatment groups after 24 h ($P > 0.05$). A lower odor and color parameter score for the control group compared with the supplemented groups led to a lower SI.

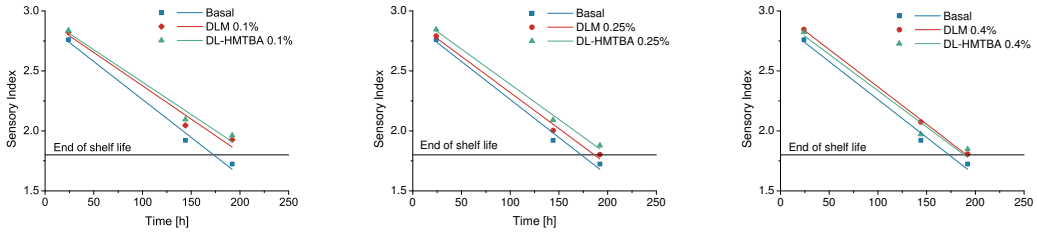

Figure 2.3.: Development of the SI and sensory shelf life of the treatment groups (n = 105 [15 per group]).

In contrast to previous studies (Liu et al., 2006), no effect on the color could be observed by Met source or concentration ($P > 0.05$). After 192 h, differences in the development of sensory characteristics between the groups could be observed. The deteriorative changes of odor and texture of the basal group occurred faster than the supplemented groups, which are reflected by the lowest sensory scores. DLM and DL-HMTBA showed no difference in the development of the sensory index at the beginning, during or at the end of storage, which is reflected in comparable shelf life times (8 to 9 d) between the supplemented groups. For the control group, the development of the SI emphasizes the rapid sensory quality loss with a sensory end of shelf life after 176 h (7 d) (Table 2.2), despite having the lowest microbiological counts at the end of storage. This could be caused by chemical spoilage processes and a lower stability against oxidative stress due to a Met deficiency (Del Vesco et al., 2015). Additionally, the specific spoilage organism is potentially inhibited by the lower drip loss and the low pH.

The sensory shelf life of commercially produced fresh poultry stored at 4°C is around 6 to 8 d (Bruckner et al., 2012b). The slight prolongation of shelf life during this trial is probably caused by the non-commercial slaughtering methods. In comparison to a commercial broiler meat production in facilities with a high throughput, microbial load and composition of the air as well as processing equipment is different (Lues et al., 2007), which led to comparable low initial microbial counts on the meat, a different microflora and different species of *Pseudomonas* spp., respectively. Additionally, the supplementation of dietary Met leads to a lower lipid peroxidation (Swennen et al., 2011; Zeng et al., 2015), which is one major aspect during the spoilage process and the volatilization of off odors (Ladikos and Lougovois, 1990). Positive effects on lipid peroxidation may have led to better evaluations for the odor score of the SI and thus to a longer shelf life for the supplemented groups in comparison to the basal group.

During sensory investigations, it became noticeable at the beginning of the trial that

two-thirds of the samples expressed White Striping (WS), a breast myopathy. The occurrence of this phenomenon was distributed over all treatment groups, with a higher incidence in animals with heavier filets and faster growth rates (data not shown), as it is also reported in recent studies (Kuttappan et al., 2012, 2013; Petracci et al., 2015). As one of several meat myopathies, WS is reported as a condition caused by genetic selection with the main focus on fast production, high growth rates and increased meat yields (Dransfield and Sosnicki, 1999; Petracci et al., 2013; Mudalal et al., 2015; Kuttappan et al., 2016). As a consequence, meat quality is affected, but the detailed effects on important shelf life parameters are not known. Nevertheless, since WS was not the focus of this trial, further investigations are necessary to clarify the effect of the diet on the occurrence and severity of WS.

2.5. Conclusions

In the present study, the effect of dietary Met supplementation on the quality parameters and shelf life of fresh poultry were investigated. No significant differences between DLM and DL-HMTBA supplementation could be shown for the investigated meat quality parameters. The supplementation of Met led to heavier fillets, a higher pH-value and a longer sensory shelf life in comparison to the basal group. In contrast, the microbial loads at the end of storage were lower for the basal group, indicating a chemical spoilage process for the basal and a microbial spoilage for the supplemented groups although all the values were in the acceptable range. For the drip loss, a significant correlation between drip loss and DL-HMTBA treated birds could be shown. Further investigations are needed to clarify the detailed relationship between Met concentration and drip loss as well as microbial growth and shelf life. In conclusion, the influence of Met supplementation on the quality and shelf life of broiler meat is complex. Thus, more research should be done for elaborating detailed recommendations for the supplementation of Met sources and concentrations in relation to meat quality and shelf life. Finally, further studies should be conducted under commercial slaughtering and processing conditions to consider a possible influence of factors like the cooling process or industrial slaughterhouse flora, too.

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3. Assessment of Meat Quality and Shelf Life from Broilers Fed with Different Sources and Concentrations of Methionine

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3.1. Abstract

A trial with different concentrations of DL-methionine (DLM) and DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA) in broiler feed was performed to investigate their effect on the meat quality parameters and the shelf life of breast filet. In total, filets from 210 male broiler chickens (Ross 308) were tested in seven groups with 30 animals each. Three different concentrations (0.04, 0.12, and 0.32%; on an equimolar basis) of either DLM or DL-HMTBA were added to a basal diet, summing up to seven treatment groups. After slaughter, filets were packed aerobically and stored at 4°C. The investigated parameters comprised measurements of microbial as well as physicochemical parameters, such as pH, drip loss, cooking loss and color measurements. Additionally, sensory investigations were conducted and shelf life was calculated. Mean pH-values were between 6.1 and 6.4. Drip loss values were low, with mean values below 0.4%. The cooking loss ranged between 22% and 28% on average. The filets showed a normal initial microbial quality (2.5 $\log_{10}$ cfu/g) and spoilage process with microbial counts of 8.5 $\log_{10}$ cfu/g at the end of storage. The study revealed a significant influence of methionine supplementation on the quality of broiler breast meat in comparison with the basal group. Methionine supplementation led to higher pH-values and a higher water binding. Higher concentrations of methionine had a positive influence on the water holding capacity by lowering the cooking loss. The L*-value showed a significant negative correlation to the methionine concentration supplemented. No differences in physicochemical as well as sensory parameters could be detected between both methionine sources. The filets showed a normal sensory spoilage process and a shelf life of 6 d. White Striping was positively correlated to filet weight as well as color values and significantly affected the purchase decision, the sensory investigation and thus the shelf life of the samples.

3.2. Introduction

As the first limiting amino acid in grain and soybean meal diets, methionine (Met) is widely-used as a supplement in broiler feed in poultry production (Baker, 2006; Bunchasak, 2009). Two common synthetic Met sources are DL-methionine (DLM) and DL-methionine hydroxy analogue free acid (DL-HMTBA). Positive effects of both Met sources on weight gain are undisputed (Garlich, 1985; Wallis, 1999), but differences in the bioavailability and efficacy have been discussed in several studies (Huyghebaert, 1993; Mandal et al., 2004; Meirelles et al., 2003; Sangali et al., 2015; Sauer et al., 2008;

Elwert et al., 2008; Lemme et al., 2002; Hoehler et al., 2005; Vazquez-Anon et al., 2006; Vedenov and Pesti, 2010). The different structure of both molecules results in diverging absorption, metabolic pathways and physiologic transformation (Richards et al., 2005; Zhang et al., 2017). Besides the positive effect of Met on the growth and feed conversion rate of the animals, Met supplementation is known to increase the breast and leg meat yield of broilers, irrespective of the Met source used (Daenner and Bessei, 2003; Liu et al., 2006; Motl et al., 2005; Yao et al., 2006; Zhan et al., 2006). Additionally, the abdominal fat content is reduced (Liu et al., 2006; Mandal et al., 2004; Wallis, 1999) as well as the absolute fat content in the filet (Aksu et al., 2007). The influence of Met supplementation on lipogenesis is discussed as a potential support to producing lean poultry meat (Fouad and El-Senousey, 2014; Takahashi and Akiba, 1995). However, positive effects on the nutritional value of poultry meat remain controversial, since Liu et al. (2006) reported no effect on the absolute content of fat or crude protein. In several studies, it was shown that Met supplementation has the ability to influence important quality parameters of the meat. Increasing Met concentrations in the diet elevates the pH of broiler meat (Aksu et al., 2007; Albrecht et al., 2017). The meat color is affected by lowering the L* and b*-value (Aksu et al., 2007; Wang et al., 2009). Hence, the coloration of filets and thighs is judged superior in comparison to Met deficient diets (Liu et al., 2006). These intrinsic characteristics, such as the structure, nutrient content, pH or water availability have a large impact on the spoilage process of the meat (Gill, 1983; Huis in't Veld, Jos H.J., 1996), especially poultry meat (Bruckner et al., 2012). Generally, changes in the physicochemical properties of poultry meat might lead to differences in the microbial spoilage process. Indeed, a relationship between Met supplementation and bacterial counts on the meat surface has been reported, but the results are contradictory. While Aksu et al. (2007) detected lowering bacterial counts with increasing dietary Met concentrations, Albrecht et al. (2017) found a supporting effect on microbial growth. In fact, there is a lack of studies focusing on the relationship between the microbial growth and the shelf life of the meat and Met supplementation. In addition to the effect on the physicochemical properties of meat, the lipid oxidation during storage can be decreased by the increase of supplemental Met (Berri et al., 2012). The diets supplemented with Met are reported to have a positive effect on lipid oxidation of the investigated broiler filets (Aksu et al., 2007; Takahashi and Akiba, 1995; Wang et al., 2009). Lipid oxidation is a major factor during the spoilage of fresh meat and influences consumer acceptance of the product (Ladikos and Lougovois, 1990). Furthermore, a positive effect on the sensory acceptance in comparison to the control group was reported

by Albrecht et al. (2017). However, it became evident during the laboratory trial, that a large number of samples expressed White Striping (WS), a breast myopathy. WS is reported to be especially frequent in heavier filets (Kuttappan et al., 2012, 2013; Petracci et al., 2015). Genetic selection with a focus on fast production, high growth rates and enlarged meat yield is supposed to increase the potential for meat defects such as WS and wooden breast (Dransfield and Sosnicki, 1999; Kuttappan et al., 2016; Mudalal et al., 2015; Petracci et al., 2013). Meat quality parameters are affected by Met supplementation. But up to now, there is a lack of studies describing the relationship between breast myopathies and typical spoilage parameters as well as shelf life. Additionally, the ability of Met supplementation to increase growth rates and meat yield of broiler and a possible influence on the prevalence on WS has not been investigated yet. Up to now, there are hardly any published studies focusing on the correlation between dietary Met supplementation and physicochemical parameters, like microbial load, breast myopathies and typical sensory parameters of commercially produced broiler meat. Thus, the objective of this study was to investigate the effect of different concentrations of liquid DL-2-hydroxy-4-methylthiobutanoic acid (DL-HMTBA) relative to DL-methionine (DLM) in chicken feed on the meat quality parameters, meat defects, spoilage process and shelf life of commercially produced breast filets.

3.3. Material and Methods

3.3.1. Study Design

Upon hatching, 800 male broiler chickens (Ross 308) with a weight of 42 g were allocated to six treatments and one basal group as control. All treatments were replicated six times with 20 birds per replication each, except for both highest Met concentrations. The highest concentrations for DLM and DL-HMTBA were replicated five times with 20 birds each. Raising and feeding was conducted at the facilities of the company feedtest (Wettin-Löbejün, Germany). The treatment groups comprised three concentrations of each Met source: DLM (MetAMINO, Evonik Nutrition & Care GmbH, Germany) and DL-HMTBA (MHA, Novus Europe SA/NV, Brussels, Belgium). The concentrations supplemented at 0.04%, 0.12% or 0.32% of either DLM or DL-HMTBA on an equimolar base to a control basal diet deficient in Met+Cys (Table 3.1). The slaughter and butchering of the broilers took place at the age of 35 d. For each treatment, 30 birds were randomly selected. The slaughtering and cooling process was conducted in a com-

mercial slaughterhouse in Jena (Gönnataler Putenspezialitäten GmbH, Germany) to simulate practical conditions. Thus, influences of a deficient cooling and processing procedure could be minimized. The samples were transported to the University of Bonn under temperature-controlled conditions in insulated boxes with cooling packs.

Table 3.1.: Feeding composition of the basal diet

Diet composition	0-10d	11-22d	23-35d	Nutrient composition %	0-10d	11-22d	23-35d
Corn, %	46.3	58.63	58.67	Crude protein	24.26	20.00	18.15
Soybean meal, 48 %CP	26.81	24.80	20.89	AMEn, MJ/kg	12.70	12.97	13.31
Peas, %	10.00	5.19	10.00	AMEn, kcal/kg	3.035	3.100	3.180
Corn gluten meal, 60%CP CP, %	8.87	3.66	2.40				
Soybean oil, %	3.09	3.31	4.24				
Monocalciumphosphate, %	1.71	1.54	1.23				
Limestone (CaCO3), %	1.64	1.35	4.24	SID Lys	1.29	1.10	1.00
Premix Blank Poultry, %	0.50	0.50	0.50	SID Met	0.34	0.27	0.24
L-Lysine, %	0.37	0.28	0.23	SID Cys	0.30	0.26	0.23
Sodium bicarbonate, %	0.24	0.22	0.00	SID M+C	0.64	0.53	0.47
Salt (NaCl), %	0.18	0.21	0.35	SID Thr	0.82	0.71	0.65
Choline Cloride 60%	0.15	0.13	0.15	SID Trp	0.21	0.18	0.16
ThreAMINO	0.08	0.09	0.08	SID Arg	1.32	1.14	1.05
ValAMINO (L-Valine)	0.05	0.07	0.07	SID Ile	0.91	0.76	0.71
L-Isoleucine	0.00	0.00	0.03	SID Leu	2.17	1.67	1.47
				SID Val	1.02	0.88	0.80

The filets were individually placed in polypropylene trays with lids and were stored at 4°C in low-temperature high precision incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan). The storage temperature was monitored by data loggers (ESCORT JUNIOR Internal Temperature Data Logger, Escort, New Zealand) every 3 minutes. Laboratory investigations started 24 h postmortem and comprised weighing of the filets, measurements of physicochemical parameters (pH, drip loss, cooking loss) as well as microbial investigations. This investigation block was repeated at 192 h of storage. Color measurements were conducted after 24 h, 168 h and 216 h. Sensory investigations, including purchase decision and breast myopathies like WS, covered the whole storage period with six investigation points in intervals of 24 h to 48 h.

3.3.2. Physicochemical Analysis

Meat pH-Value

The surface pH of the filets was measured using two portable surface pH-meters (pH 8011, Peter Bock Umwelttechnik, Gersfeld, Germany; GPH114, GHM Messtechnik GmbH Standort Greisinger, Regenstauf, Germany). Two measurements were performed for each meat sample by placing the electrode onto the meat surface and an average pH-value was calculated.

Measurement of the Drip Loss

The measurement of drip loss and cooking loss was conducted to characterize the water binding capacity of the meat samples (Trout, 1988). Drip loss measurements of the breast filets were conducted after 24 h and 192 h of storage. After being packed in plastic bags, meat samples were hung on hooks through their thickest part for 24 h in a 4°C incubator. Samples were weighed before and after hanging. Drip loss was calculated as the loss in weight, corrected for size and expressed as a percent (equation 3.1).

$$D_L = \frac{m_1 - m_2}{m_1} \cdot 100\% \tag{3.1}$$

Where D_L is drip loss [%], m_1 is mass before hanging and m_2 is mass after hanging.

Measurement of the Cooking Loss

Measurements of the cooking loss were performed 24 h after slaughter. A sample of around 3 x 5 cm was taken with a scalpel from the caudal end of the filets. The samples were weighed and packed separately in autoclave bags. The samples were cooked at 80°C in a water bath (Memmert, Schwabach, Germany) until the core temperature of the filets reached 72°C. The core temperature was measured with a food core thermometer (Testo, Lenzkirch, Germany). A second weighing was conducted after cooking and the cooking loss was calculated as the loss in weight, corrected for size and expressed as a percent (equation 3.1).

Color Measurements

Color measurements were conducted 24 h, 168 h and 216 h of storage with a large view spectrophotometer (MiniScan EZ 4500L, HunterLab, Murnau). The device works with a wavelength between 400 nm and 700 nm and a 45°/0° geometry. The CIE 1976 Lab

scale was used, measured with D65 illuminant (6500 K daylight). The filets were placed on cooled glass plates for the measurements. The L*a*b* and C, h° values were recorded for each filet at three sample points to get a representative evaluation of the samples. Only the L*a*b*-values were selected for analysis and measurements were averaged for each filet.

3.3.3. Microbiological Analysis

For the microbiological analysis, 25 g of surface meat tissue, with a size of 3.5 x 7 x 0.5 cm, was aseptically taken using a sterile scalpel. The sample was transferred to a filtered, sterile stomacher bag and filled with 225 ml of saline peptone diluent (0.85% NaCl with 0.1% peptone Saline-Tablets, Oxoid BR0053G, Cambridge, United Kingdom). The samples were mixed with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60 s. Tenfold dilutions of the homogenate were prepared in saline peptone diluents. *Pseudomonas* spp. (Pse) were detected by spread plate technique on *Pseudomonas* agar with Cetrimide-Fucidin-Cephalosporine selective supplement (CFC, Oxoid, Cambridge, United Kingdom). Plates were incubated at 25°C for 48 hours.

3.3.4. Sensory Investigation

Sensory investigations comprised the sensory evaluation of the spoilage process to determine the shelf life of the samples, an assessment of meat failures such as WS and the purchase decision. All sensory investigations were performed by a trained sensory panel including six panelists after 24 h, 72 h, 120 h, 168 h, 192 h, and 216 h. The training of panelists was conducted during former trials and exercise courses prior to the main trial.

Purchase decision

Before each sensory evaluation, the sensory panel evaluated the samples for the trait Purchase decision. Based on overall visual appearance, each panelist chose whether they would purchase each sample or not. In order to avoid biased perceptions of the samples (i.e. through odor), this answer was given prior to the other sensory evaluations. The results of all panelists were then averaged and expressed as a percent for each sample. Additionally, the sensory panel noted demerits visually apparent on the meat surface such as hematoma, cuts caused by processing failures or color anomalies. Demerits went into the analysis as total number for every filet.

Sensory Index

For each sample, the characteristics color, odor and texture were assessed via a graded three-point-scoring system, with three meaning fresh and high quality and one meaning unacceptable. The Sensory Index (SI) was calculated as a weighted average with the following equation 3.2

$$SI = \frac{2 \cdot O + 2 \cdot C + T}{5} \tag{3.2}$$

Where SI is the Sensory Index, the O is the odor, C is the color and T is the texture.

According to the scheme, the product is spoiled when the SI reaches the level of 1.8. The SI was plotted as a function of time and fitted to a linear model. Thus, the shelf life of each sample was calculated by equation 3.3 as follows (Kreyenschmidt, 2003):

$$SL = \frac{1.8 - a}{b} \tag{3.3}$$

Where SL is shelf life, a is the intercept of the linear model and b is the slope of the linear model.

Assessment of White Striping

Following the sensory evaluation, the appearance of WS was evaluated by the sensory panel with a 3-point-scoring system. A score of "0" means no WS, "1" means medium WS and "2" means severe WS (Figure 3.1). The results of all panelists were then averaged for each sample.

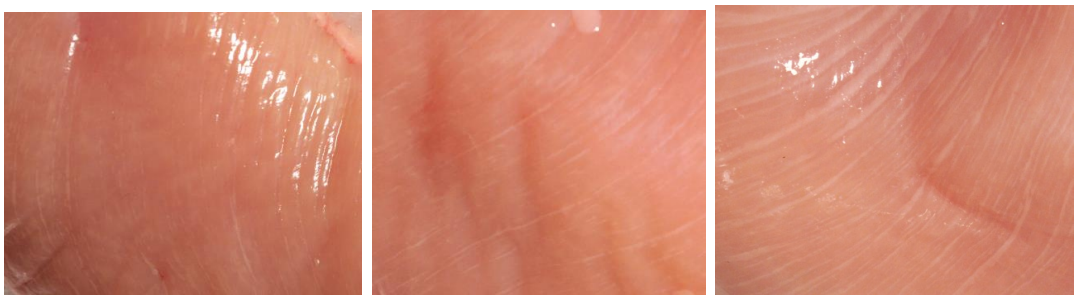

Figure 3.1.: White striping: 0—no WS (a), 1—medium WS (b), and 2—severe WS (c)

3.3.5. Data Analysis and Statistics

Data were tested for normal distribution and homoscedasticity. Since data did not meet the conditions for parametric statistical tests, nonparametric methods for statistical tests were used. For illustrating data distribution, boxplots were used displaying median as well as first and third quartiles of data. Differences between groups were tested with the Kruskal-Wallis-Test. In the event of significant differences, pairwise comparisons were performed with the Dunn-Bonferroni-Test to test differences between individual groups. Correlations were tested with Spearman's Rank Correlation Test and the correlation coefficient k was computed (with $k<0.4$ meaning a low correlation, $0.4<k<0.6$ meaning a medium correlation and $k>0.6$ meaning a high correlation). Test results are marked with ($p<0.05$) for significant and ($p<0.001$) for highly significant differences or correlations. To explore the influence of several explanatory variables on the response variable 'purchase decision', a multiple linear model was calculated. Predictors with a significant influence on the response variable were used to calculate and plot a second degree response surface model. Data analysis was conducted with statistical software R 2.15 (R Development Core Team). Additionally, SPSS Statistics 23 (IBM Corp. 1989,2013, New York, USA) and OriginPro 8G (OriginLab Corp., Northampton MA, USA) were used.

3.4. Results and Discussion

The investigated samples had filet weights between 47.2 g and 288.75 g with mean values between 83.17 g and 240.20 g (Table 3.2). The filet weight was significantly correlated to the Met concentration (k:0.914, $p<0.001$, Table 3.3). The difference in weight gain between concentration groups was significant $p<0.001$. The results of this study are in accordance with former studies reporting a significant increase of breast meat yield in Met supplemented broilers (Elwert et al., 2008; Zhan et al., 2006; Yao et al., 2006; Daenner and Bessei, 2003; Motl et al., 2005; Liu et al., 2006).

The pH-value of the filets ranged between 6.20 and 6.35 at the first investigation point (Table 3.2). For poultry filets, the typical range of pH-values 24 h after slaughter is between 5.6 and 5.9 (Debut et al., 2003; Garcia et al., 2010), but may also range up to 6.02 (Bruckner et al., 2012). A storage-related increase of meat pH up to values between 6.43 and 6.58 was observed and can be explained by an accumulation of metabolites of the growing microorganisms (Nychas et al., 2008). The basal group showed significantly lower pH-values at the beginning of storage ($p<0.05$), indicating that Met supplemen-

Table 3.2.: Meat quality parameters in breast muscle of chickens fed based on different Met sources and concentrations

	Basal	DLM 0.04	DLM 0.12	DLM 0.32	DL-HMTBA 0.04	DL-HMTBA 0.12	DL-HMTBA 0.32
Weight	83.17^{a}	148.80^{bc}	205.25^{def}	240.20^{dg}	134.95^{ab}	183.27^{ce}	233.37^{fg}
	±17.53	±20.37	±25.37	±20.79	±20.48	±22.30	±24.64
Pse_{24}	2.07^{a}	1.61^{a}	1.79^{a}	2.08^{a}	1.67^{a}	2.17^{a}	1.72^{a}
$[cfu/g]$	±1.00	±0.55	±0.51	±0.89	±0.66	±0.59	±0.65
Pse_{192}	8.29^{a}	8.51^{b}	8.17^{a}	7.82^{ab}	8.08^{a}	7.89^{ab}	7.96^{a}
$[cfu/g]$	±0.52	±0.44	±0.39	±0.53	±0.43	±0.41	±0.48
pH_{24}	6.16^{a}	6.30^{b}	6.30^{b}	6.27^{b}	6.23^{ab}	6.28^{b}	6.32^{b}
	±0.17	±0.15	±0.15	±0.19	±0.21	±0.22	±0.18
DL_{24}	0.402^{a}	0.217^{ab}	0.175^{b}	0.216^{b}	0.295^{ab}	0.213^{ab}	0.192^{b}
	±0.34	±0.12	±0.10	±0.20	±0.27	±0.10	±0.17
CL	28.21^{a}	25.86^{ab}	22.97^{ab}	22.24^{ab}	26.90^{ab}	24.53^{ab}	22.13^{b}
	±7.56	±6.43	±5.16	±5.61	±6.26	±5.14	±4.91
L^*_{24}	57.61^{ab}	58.80^{a}	57.21^{ab}	55.68^{b}	58.14^{a}	58.33^{a}	56.60^{ab}
	±2.14	±2.55	±2.94	±2.67	±2.57	±2.59	±3.2
a^*_{24}	7.2^{a}	6.88^{ab}	6.8^{ab}	6.61^{ab}	7.03^{ab}	6.62^{ab}	6.32^{b}
	±0.95	±0.91	±1.08	±1.08	±1.09	±0.89	±0.73
b^*_{24}	14.93^{a}	15.88^{ab}	15.65^{ab}	14.87^{ab}	15.8^{ab}	15.72^{ab}	15.14^{b}
	±1.18	±1.31	±1.57	±1.76	±1.82	±1.72	±1.61
SI_{24}	2.79^{a}	2.70^{b}	2.70^{b}	2.72^{b}	2.72^{ab}	2.72^{ab}	2.74^{ab}
	±0.07	±0.10	±0.11	±0.11	±0.11	±0.11	±0.10
Shelf	142^{a}	133^{b}	137^{b}	134^{b}	139^{b}	138^{b}	138^{b}
life $[h]$	±12.05	±9.67	±8.83	±10.21	±11.64	±11.41	±9.16

Pse: *Pseudomonas* spp., DL: drip loss, CL: cooking loss, DLM: DL-methionine, DL-HMTBA: DL-methionine hydroxy analogue free acid

tation elevates the ultimate meat pH as reported in former studies (Aksu et al., 2007; Albrecht et al., 2017). The positive effect of Met supplementation on stress-related genes and protective cellular mechanisms against oxidative stress might explain higher pH-values (Fang et al., 2002; Luo and Levine, 2009; Del Vesco et al., 2013). Meat pH is strongly influenced by stress during the pre-slaughter and slaughter processes, meaning that high stress leads to an accumulation of lactic acid in the muscle, resulting in a lower ultimate meat pH (Berri, 2000; Debut et al., 2003). Thus, the comparatively high pH-values during this trial are possibly caused by an enhanced metabolic stress resistance by Met supplementation, whereas no significant difference was observed between Met sources ($p>0.05$). The absence of lactic acid in muscle tissue, leading to a higher meat pH, is associated with a high water binding capacity of the myofibrillar proteins (Huff-Lonergan and Lonergan, 2005). The drip loss of the breast filets ranged between 0.18% and 0.40% 24 h after slaughter (Table 3.2). The basal group had a significantly

higher drip loss than both the DLM 0.12 and 0.32 (p=0.03) and the DL-HMTBA 0.32 groups (p=0.035), but there was no difference between Met sources (p>0.05). In general, drip loss values were lower in comparison to other studies with mean drip loss values between 0.42% and 3.32% (Le Bihan-Duval et al., 2008; van Laack et al., 2000; Woelfel et al., 2002). Additionally, the supplementation of Met showed a tendency to lower the drip loss of the breast filets, which was confirmed by a significant but low negative correlation between Met concentration and drip loss 24 h after slaughter (k:-0.205, p=0.003, Table 3.3). This contradicts findings of former studies reporting a positive relationship between increasing Met concentrations and drip loss or moisture content (Albrecht et al., 2017; Liu et al., 2006). Reduced drip loss values in comparison to the basal group are in accordance with the effects of Met supplementation on elevated meat pH and former studies, which reported lowering drip loss values with increasing Met concentration (Wang et al., 2009). However, differences in drip loss between all groups seem to fade during storage. After 192 h, drip loss showed a slight increase to mean values between 0.23% and 0.30% (Appendix Table A.1.1, p. 136) and a convergence between the groups, which is probably caused by the spoilage process and the enzymatic and chemical deterioration of the meat. Drip loss values at 192 h showed no differences (p>0.05) between treatment groups and no significant correlation to the Met concentration. The mean cooking loss of the breast filets varied between 22% and 28% and no significant difference between the Met sources was observed (Table 3.2). The cooking loss of the 0.32% Met supplementation was significantly lower than both the basal group (p=0.004) and the 0.04% Met supplementation (p=0.022). The cooking loss was negatively correlated to the Met concentration, irrespective of the treatment groups (k=-0.377, p<0.001). In general, the water binding capacity of meat is influenced by the genotype, a fast muscle growth, pre-slaughter stress as well as the conditions during slaughter and processing (Debut et al., 2003; Huff-Lonergan and Lonergan, 2005). An influence of the diet composition on the water holding capacity of meat has been reported (Berri et al., 2008; Downs et al., 2000; Jiang et al., 2009; Young et al., 2004). Met supplementation seems to improve the water binding capacity by lowering drip loss and cooking loss values, independent of the Met source supplemented. Nevertheless, the information on the effect of Met supplementation on water binding capacity of meat is inconsistent and further research is needed to clarify the relationship.

The data of the color measurement showed a high variation and broad overlap between groups (Figure 3.2).

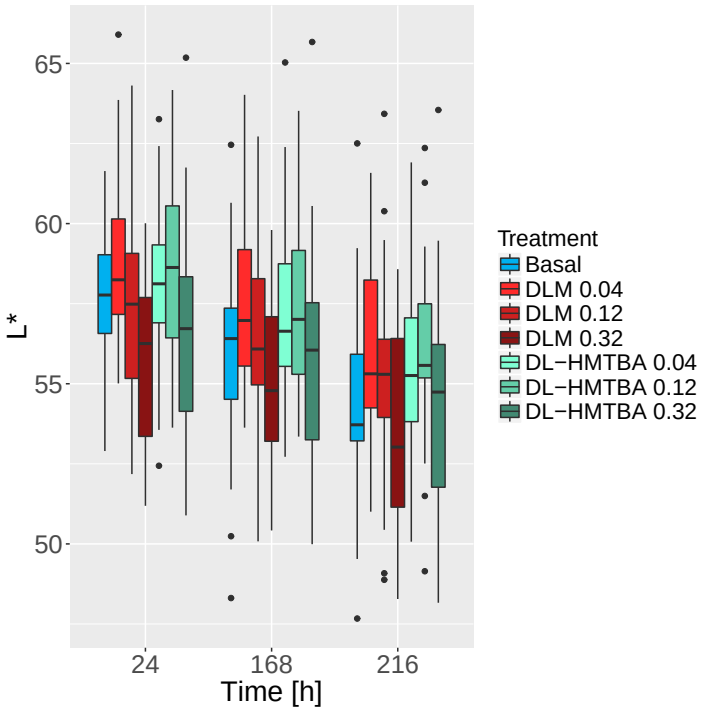

Figure 3.2.: L*-values of breast filets (n=209)

In general, increasing Met concentration led to lower L*-values, lower a*-values and higher b*-values, meaning that the filets appear darker, with a lesser red and higher yellow portion. Mean L*-values ranged between 55.68 and 58.8 at the first investigation point at 24 h (Table 3.2). L*-values showed a significant decline during storage ($p<0.01$). Samples showed significant differences in the L*-value between the highest Met concentration and the lowest as well as medium concentration ($p<0.05$). The color measurements conducted in this study revealed that poultry filets were generally lighter in comparison to the optimal color for poultry filets stated in former studies. In general, optimal L*-values for poultry are given as $47\leq L\leq 53$, taking into account the factors affecting meat color such as animal and process specific factors (Allen et al., 1998; Barbut, 1997; Petracci et al., 2004; Woelfel et al., 2002). Normally, higher L*-values, above 56 (Petracci et al., 2004) or 59 (Woelfel et al., 2002), are judged as pale, soft and exudative (PSE) meat. But, since the samples investigated in this study expressed high pH-values and a high water binding capacity, the meat cannot be judged as PSE meat. The broad occurrence of WS in the current study can be an explanation for high L*-values, since L*-values are significantly correlated to WS (k:0.354, $p<0.001$, Table 3.3). Additionally, the color of meat is strongly influenced by genetic selection, slaughtering conditions and

diet, which might be a further reason for higher L*-values (Berri et al., 2001; Fletcher, 2002; Froning, 1995; Mugler and Cunningham, 1972). The a*-values ranged from 6.61 to 7.20 between all treatment groups at the beginning of storage (Table 3.2). There was no change observed for the a*-values during storage. No significant differences between the Met sources could be observed, but the basal group showed significantly higher values than the 0.32% DL-HMTBA groups ($p<0.024$). Additionally, a*-values showed a significant negative correlation to the Met concentration as well as the L*-values, but the effect was only observed at the first investigation point at 24 h. The mean b*-values ranged between 14.87 and 15.88 at the beginning of storage. There was a significant negative correlation to the Met concentration with the highest b*-values measured at the lowest supplementation levels. Therefore, higher Met concentrations led to lower L*- and a*-values and higher b*-values. Lower L*-values under the effect of Met supplementation have been reported before (Aksu et al., 2007; Wang et al., 2009), but the results of a*- and b*-values contradict findings of Liu et al. (2006) who reported a 'superior meat color'.

Table 3.3.: Spearman's ρ correlation of meat quality parameters 24 h after slaughter

	Met conc.	Weight	Pse	pH	Drip Loss	Cooking Loss	L	a	b	SI	Shelf life
Weight	***.914***										
	.000										
Pse	.051	.012									
	.609	.906									
pH	**.176**	***.267***	.103								
	.011	***.000***	.301								
Drip	***-.205***	***-.257***	-.036	***-.209***							
Loss	***.003***	***.000***	.719	***.002***							
Cooking	***-.377***	***-.359***	.115	**-.214**	.232						
Loss	***.000***	***.000***	.257	***.029***	**.018**						
L	***-.250***	***-.239***	-.134	***-.308***	***.260***	***.357***					
	.000	***.000***	.180	***.000***	***.000***	***.000***					
a	***-.246***	**-.143**	.019	-.114	.072	.107	-.104				
	.000	**.038**	.852	.099	.302	.279	.132				
b	-.085	-.039	.011	***-.187***	.127	.079	***.533***	***.318***			
	.220	.575	.910	***.007***	.066	.424	***.000***	***.000***			
Sensory	**-.147**	**-.158**	-.087	-.018	-.078	.029	***-.346***	-.001	***-.443***		
Index	**.034**	**.022**	.384	.794	.261	.770	***.000***	.991	***.000***		
Shelf	-.094	-.042	**-.196**	**.151**	-.084	**-.217**	***-.447***	.038	***-.380***	***.579***	
life	.174	.546	**.049**	**.029**	.228	**.027**	***.000***	.581	***.000***	***.000***	
WS	**.159**	***.207***	-.116	***.194***	-.036	-.087	***.354***	.131	***.343***	***-.442***	***-.364***
	.021	***.003***	.244	***.005***	.609	.381	***.000***	.058	***.000***	***.000***	***.000***

The initial bacterial contamination of the samples showed mean values between 1.61 $\log_{10}$ cfu/g and 2.17 $\log_{10}$ cfu/g (Table 3.2). For industrial slaughter, these microbial loads are low in comparison to other studies, which reported mean bacteria numbers of 2.9 $\log_{10}$ cfu/cm^2 (Sahar and Dufour, 2014), 3.7 $\log_{10}$ cfu/g (Raab et al., 2008), 3.8 $\log_{10}$ cfu/g (Vasconcelos et al., 2014) and 4.1 $\log_{10}$ cfu/g (Bruckner et al., 2012) after slaugh-

ter. However, the initial count of industrially slaughtered poultry can vary depending on slaughter and hygienic conditions during processing (Lues et al., 2007). There was no difference in initial bacterial count between the different treatment groups ($p>0.05$) after 24 h, indicating that all samples showed a comparable initial contamination by microorganisms. After 192 h storage, the mean microbial counts of *Pseudomonas* spp. ranged between 7.82 $\log_{10}$ cfu/g and 8.51 $\log_{10}$ cfu/g (Table 3.2). The microbial acceptance level of *Pseudomonas* spp. is 7.5 $\log_{10}$ cfu/g (Bruckner et al., 2012), which was exceeded by 88% of the samples. *Pseudomonas* spp. counts were negatively correlated to DLM concentration (k:-0.339, p=0.001, Table 3.3). This is contradictory to findings of our former study where results pointed to a positive correlation between Met concentration and microbial counts, irrespective of the Met source (Albrecht et al., 2017). An effect of Met on lowering the bacterial counts on meat was also reported by (Aksu et al., 2007), but no particular explanation was given. Since the relationship between Met supplementation and the growth of *Pseudomonas* spp. could not be clarified, further studies are needed to investigate if these effects are a result of causal connections or a statistical bias. The sensory investigations revealed that the samples showed a normal initial meat quality upon arrival at the laboratory. The mean SI ranged between 2.7 and 2.8, 24 h after slaughter. The SI was evaluated significantly worse for the DLM and DL-HMTBA groups than for the basal group at the beginning of storage (Table 3.2), which is mainly caused by differences in the color evaluation. Additionally, a low negative correlation between the SI and the Met concentration was detected (k=-0.147 , p=0.034). The SI showed a linear decline with time with a similar gradient for all treatment groups, meaning a similar speed of the spoilage process for all filets. The mean sensory shelf life of the treatment groups ranged between 133 h and 142 h (Table 3.2). For commercially produced poultry a shelf life of 6 days has been reported before (Bruckner et al., 2012). The shelf life in the present study showed a significant difference between the DLM and the basal group (p=0.01), but the difference is below 24 h and thus judged not relevant for the poultry industry. For SI and shelf life, there were no significant differences detected between the DLM and the DL-HMTBA group. The evaluation of the purchase decision revealed significant differences between the treatment groups. The basal group was evaluated significantly better at all investigation points ($p<0.05$). Linear modeling revealed that the parameters WS and color (visual assessment) had a significant influence on the purchase decision in the first 72 h of storage.

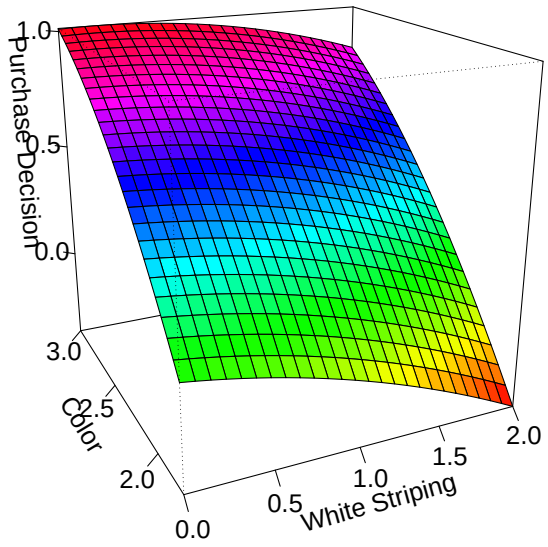

Figure 3.3.: Influence of color and White Striping on the purchase decision (t=24 h & 72 h)

In contrast, Met source or size of the filets had no influence on the purchase decision (Appendix, Tables A.1.2 and A.1.3, p. 135). WS is supposed to affect the consumer acceptance of raw meat (Kuttappan et al., 2016; Sanchez Brambila et al., 2016). The relationship between color, WS, and purchase decision is shown in Figure 3.3. At the investigation points 24 h and 72 h, the best purchase decision was achieved for filets with the highest color scores and low scores for WS. WS led to less positive purchase decisions, even if the color of the filets was optimal. As a consequence, filets with a low rating for color and higher occurrence of WS were rejected by the sensory panel even if the spoilage level was not yet reached. With proceeding storage, the spoilage process became apparent and led to low ratings for purchase decision. Over 90% of all samples were rejected at 168 h when the meat was spoiled. For both Met sources, less than 10% of filets were rated with "no WS". In contrast, over 30% of the filets of the basal group showed "no WS" which could be due to suboptimal Met supplementation below typical industrial conditions and lower growth rate. White Striping occurred in most of the samples with dominance on "medium WS" (Figure 3.4).

The occurrence or severity of WS is weakly correlated with the concentration of Met

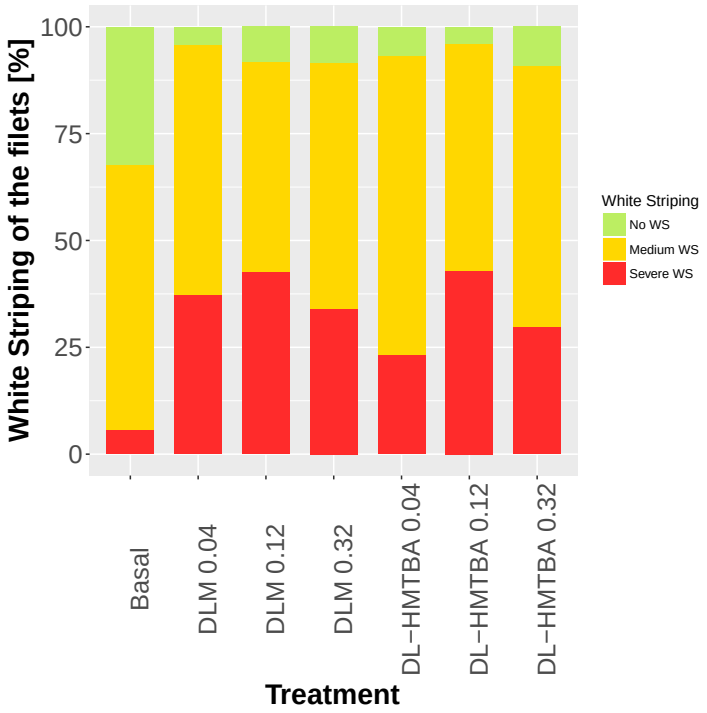

Figure 3.4.: Occurrence of White Striping in breast filets

supplementation (k=0.159, p=0.021) and had a negative influence on the purchase decision. The correlation is weak, because severe White Striping was most distinct in the medium Met concentration. There was no significant difference in the severity of WS between the DLM and the DL-HMBTA groups. However, both Met groups expressed significantly more WS than the basal group. WS is a breast myopathy probably caused by the increased growth rate of animals in the modern poultry industry (Kuttappan et al., 2012; Petracci et al., 2015). One-sided genetic selection for higher growth rates and meat yield has been linked to muscle abnormalities in earlier studies (Dransfield and Sosnicki, 1999). Even if several investigations could not relate genetic selection of commercial broiler lines to a negative impact on meat quality (Berri et al., 2001; Le Bihan-Duval et al., 2008), WS was observed significantly more often in heavier and fast growing birds of modern broiler lines (Kuttappan et al., 2013; Petracci et al., 2013, 2015; Russo et al., 2015). The dietary supplementation of lysine is reported to have an influence on protein metabolism and induces the occurrence of WS (Cruz et al., 2017). In addition to nutritional factors, a connection to changed metabolic mechanisms, proliferation of connective tissues, genetic predisposition, or a combination of these parameters, are discussed (Baldi et al., 2017). The detailed mechanisms causing WS are still not

clear (Kuttappan et al., 2016). Thus, the nature of the effect of Met supplementation on the occurrence of WS, whether it is causal or rather a side effect, and its interaction with other factors, are currently unclear. Further investigations are needed to clarify these effects.

3.5. Conclusion

The supplementation of Met at three different dietary levels showed a significant effect on the meat yield and quality of broiler filets in comparison to the basal group. Met supplementation resulted in higher pH-values and showed a positive effect on water binding capacity, irrespective of the Met source used. The microbial load at the end of storage decreased with increasing Met concentration, but this effect did not lead to a relevant prolongation of shelf life. The shelf life of the filets was 6 days, which is a proper shelf life for industrially produced, aerobically stored poultry filets. Methionine supplementation was negatively correlated to L*, a*, b*-measurements, to a magnitude that was also visually noticed by the sensory panel. White striping occurred in most of the samples and was significantly correlated to filet weight. The occurrence of WS showed a low correlation to Met concentration and significantly affected the color of the samples as well as purchase decision. No specific parameter provoking WS could be identified. In summary, the effects of dietary Met supplementation on the quality and freshness of poultry meat is complex and more research is needed to clarify the relationship between dietary Met supplementations and meat quality as well as the occurrence of WS.

3.6. Acknowledgments

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4. Influence of Different Production Systems on the Quality and Shelf Life of Poultry Meat: A Case Study in the German Sector

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4.1. Abstract

Production-specific factors, such as breeding, diet, and stress, are known to influence meat quality, but the effect of different husbandry systems on the development of quality parameters and shelf life has hardly been investigated. Thus, the aim of the study was the investigation of an alternative production system based on a slow growing, corn-fed, and antibiotics-free chicken line compared with conventional poultry production. Additionally, the effect on meat quality, microbiology, and spoilage was analyzed. In total, 221 breast filets from a German poultry meat producer were investigated. Nutritional, biochemical, and cooking loss analyses were conducted on a subset of samples 24 h after storage. The rest of the samples were stored aerobically at 4°C, and the spoilage process was characterized by investigating pH, color, lipid oxidation, microbiology, and sensory attributes subsequently every two days during storage. The alternative production line showed a significantly healthier nutritional profile with a higher protein and lower fat content. Additionally, the amount of L-lactic acid and D-glucose was significantly higher than in the conventional production line. The color values differed between both production lines, with the corn-fed line displaying more yellowish filets. The lipid oxidation and microbial spoilage were not affected by the production line. The shelf life did not differ between the investigation groups and was deemed 7 days in both cases. Despite the highest severity of White Striping being observed most in the conventional production line, there was no overall difference in the incidence among groups. The purchase decision was affected by the occurrence of White Striping and showed a tendency for a higher acceptance for the alternative production line.

4.2. Introduction

To meet the growing consumption and consumer demands, poultry production underwent a remarkable development of intensification. As a result of intense selection processes, poultry breeding lines were modified for shorter generation times, enhanced animal performance, and higher meat content (Anthony, 1998). The slaughter age was halved to five weeks, while the breast meat yield was significantly increased by 10% compared with poultry production 50 years ago (Petracci et al., 2015; Rauw et al., 1998). With the selection for growth velocity, an increase of muscle failures and health issues of the animals arose (Bessei, 2006; Dransfield and Sosnicki, 1999; Julian, 1998). White Striping (WS), for example, is a muscle myopathy correlated to heavy breast

filets and fast muscle growth (Kuttappan et al., 2012b). WS has a remarkable negative impact on consumer acceptance (Kuttappan et al., 2012b, 2016). Further undesirable characteristics caused by the selection for high production efficiency are immunological, behavioral, physiological, and stress-related problems (Rauw et al., 1998).

Animal health issues were countered by increased application of antibiotics in industrial animal production, which resulted in the proliferation of microorganisms with antibiotic resistance (Silbergeld et al., 2008) with enormous impact on human health. In the context of these problems, the sustainability of poultry production is discussed increasingly, and consumer awareness is rising for animal health and welfare topics (Magdelaine et al., 2008; Leinonen and Kyriazakis, 2016; Troy et al., 2016; Castellini et al., 2012). The increasing demand for extensive production systems which are vigilant for animal welfare resulted in a growing organic sector, local certified products, and the establishment of high-quality meat lines (Magdelaine et al., 2008; Borell and Sørensen, 2004). One example for a high quality, local product is the French poultry line "Label Rouge," which was successfully introduced in the market in the 1960s and is widely accepted (Westgren, 1999). In Germany, the production of specialized corn-fed poultry lines is a similar attempt to launch high-quality meat in the market and experience a positive resonance with the customer. As the meat market reached the saturation point in Germany, meat quality as well as animal welfare and sustainability have an increasing impact on the purchase decision of the consumer. Thus, a production system was developed focusing on enhanced animal welfare, antibiotics-free, corn-based fattening, and a slow growing breed. The use of more sustainable systems, such as the proposed one, may increase consumer acceptance and the willingness-to-pay higher prices; however, any modification of the production system may also cause differences in the meat quality, nutritional parameters, and the shelf life of the final product. Several studies are conducted under controlled laboratory conditions and not in commercial production systems and thus do not fully reflect practical conditions of meat production.

Thus, the focus of this case study was the comparison of two commercial production lines regarding typical meat nutritional and quality parameters, typical defects (such as WS), and the shelf life of the products.

4.3. Material and Methods

4.3.1. Study Design

The investigation focused on the characterization of two different industrial production lines: conventional and alternative, of a German poultry producer. For the alternative production line, the producer recently changed breeds for a new slow growing race showing optimized muscle growth within the prolonged production time. Additionally, detailed information on feedstuff ingredients is not provided due to confidentiality clauses.

Characteristics of the alternative production line were as follows: the used race was the slow growing Ranger Classic at a maximal stocking rate of 32 kg/m^2 and a toy-enriched environment, such as bales of straw and boxes. The diet of the birds contained more than 50% corn. The fattening focused on a slower growth of the animals and was conducted without antibiotic medication. The birds were slaughtered after 42–45 d.

Characteristics of the conventional production line were as follows: the race Ross 308 was used at a stocking rate of 39 kg/m^2. Antibiotic medication was administered when required. The birds were fed with a grain-based diet and slaughtered after 30–35 d.

All animals were slaughtered and processed the same day and in the same industrial slaughterhouse. The breast filets were transported under temperature-controlled conditions to the laboratory of the University of Bonn. To investigate the influence of the production system on the nutritional value (protein, intramuscular fat, collagen, and water content) and muscle characteristics (L-lactic acid and D-glucose), a subset of the samples (n = 32) was frozen directly after arrival at the laboratory.

A total of 221 filets were investigated in two repeated storage trials to assess the development of quality parameters and shelf life. After packaging aerobically in polypropylene trays with snap-on lids, the samples were stored in high-precision low-temperature incubators (Sanyo Mir 154-PE, Sanyo Electric Co., Ora-Gun, Gunma, Japan) at 4°C for 12 d. The investigations were conducted at six repeated investigation points during storage. For each investigation point, a total of 24 alternative and 13 conventional filets were investigated. The analyzed parameters comprised physicochemical parameters (pH, drip and thawing loss, and color measurements), microbial investigations (total viable count, *Pseudomonas* spp., *Brochothrix thermosphacta*, and Enterobacteriaceae), and a sensory analysis including the assessment of WS and purchase decision (PD). The analyses were conducted on the complete filet with an excision only for microbial investigations. After all investigations were completed for an investigation point, samples were frozen

at −18°C and stored for the measurement of thiobarbituric acid reactive substances. The first analyses started 24 h after slaughter (0 h of the experiment) to characterize the initial meat quality, WS, and microbial contamination of the poultry filets. Except for cooking and thawing loss, the development of quality and microbial parameters was investigated by six repeated measurements until the end of storage at 288 h (12 d).

4.3.2. Physicochemical Parameters

Analysis of nutritional value and muscle characteristics

To investigate the influence of the production system on the nutritional value of the meat and the susceptibility of the muscle for microbial spoilage, the main nutrients, D-glucose and L-lactic acid, were analyzed for a subset of the samples. The meat samples were frozen at a fresh condition 24 h after slaughter in a −18°C freezer. Before the analyses, the samples were thawed for 24 h at 4°C. The nutritional value of the poultry filets was analyzed with near-infrared spectroscopy on 32 filets. The whole filets were processed using a food processor (Moulinex DPA 141, Groupe SEB Deutschland GmbH, Offenbach, Germany). Afterwards, they were placed in the near-infrared spectrometer (NIRS DS2500, Foss, Rellingen, Germany) and analyzed automatically. The measurements comprised intramuscular fat, protein, water, and collagen content and are stated as percentages.

Two specific enzyme test kits were used to determine the content of L-lactic acid (Biopharm 1111281035, R-Biopharm AG) and D-glucose (Biopharm 10139106035, R-Biopharm AG) with a spectral photometer (Thermo Scientific^{TM} GENESYS^{TM}, Fisher Scientific GmbH, Schwerte, Germany) on 23 filets. Sample preparation was conducted following a modified protocol of (Bruckner et al., 2012a). A standardized sample size of $4 \times 8\,\mathrm{cm}^2$ was extracted with a scalpel and processed using a food processor. 5 g of the meat paste was transferred to a beaker glass, dissolved in 35 ml Aqua Bidest, and homogenized for 5 min on a magnetic stirrer without heating. After Carrez clarification and adjusting the pH-value to 7.5–8 (testo 206-pH1, Testo, Lenzkirch, Germany) with 0.5 mol sodium hydroxide solution, the solution was transferred to a graduated flask, filled with Aqua Bidest up to 100 ml, and swiveled slightly. The solution was filtered (Whatman Filter 595 1/2, GE Healthcare Europe GmbH, Freiburg, Germany) and further processed following the instructions of the test kit. Samples were measured in repeat determination at 340 nm.

Meat pH-value

The surface pH of the filets was measured with a portable surface pH-meter (pH 8011, Peter Bock Umwelttechnik, Gersfeld, Germany). The pH-meter works with a glass electrode, specifically developed for meat surface measurements. The pH-meter is calibrated daily and checked regularly against penetration electrodes to justify correct measurements. Three measurements were performed for each meat sample by placing the electrode onto the meat surface. An average pH-value was calculated for every sample (n = 221).

Cooking and thawing loss

As a measure for the water binding capacity of the breast filets, the cooking loss and thawing loss were analyzed. The cooking loss analysis was performed on the inner filet of the meat sample at the beginning of storage (n = 36). The inner filet was detached from the sample, weighed, transferred separately into an autoclave bag, and sealed. Samples were heated in an 80°C water bath (Memmert, Schwabach, Germany) until the core temperature reached 72°C. Temperature measurements were performed with a food core thermometer (Testo, Lenzkirch, Germany) in a reference sample. After cooking, the filets were dabbed and weighed again. The thawing loss was measured by weighing the whole filet before and after freezing in an −20°C freezing room. The thawing loss was determined at the beginning (n = 22) and end of storage (n = 21). Cooking loss and thawing loss, respectively, were calculated using the following equation:

$$W_L = \frac{m_1 - m_2}{m_1} \cdot 100\% \tag{4.1}$$

where W_L is water loss [%], m_1 is mass before treatment, and m_2 is mass after treatment.

Color Measurements

The color of the filets (n = 196) was measured using a large view spectrophotometer (ColorFlex EZ 4500L, HunterLab, Murnau). The color measurement was conducted at a wavelength between 400nm and 700nm and with a 45°/0° geometry. The CIE 1976 scale was used, measured with D65 illuminant (6500 K daylight). The filets were placed on the glass surface of the measurement device. The color was measured at three sample points for each filet to get a representative evaluation of the sample. Measurement values were averaged for each sample.

Thiobarbituric Acid Reactive Substances

For the investigation of fat oxidation in the tissue, thiobarbituric acid reactive substances (TBARS) were determined by a quantitative assessment of malondialdehyde (MDA) via extraction with trichloroacetic acid (TCA) and a fluorometric measurement in a microplate reader (Synergy H1 Microplate Reader, BioTek Instruments Inc., Winooski, US). The measurement of TBARS was conducted during the second repetition of the trial on 131 breast filets (n = 14 alternative and n = 8 conventional, per investigation point). For the preparation of samples, poultry filets were thawed at 4°C for 24 h. A standardized surface of the meat tissue with a size of $4 \times 8\,\mathrm{cm}^2$ and 0.5 cm thickness was punched and homogenized with a food processor. After transferring 7 g of the meat paste to a 50 ml tube, 15 ml TCA (7.5%) was added together with ethylenediaminetetraacetic acid (EDTA, 0.1%) and propyl gallate (0.1%). Each sample was homogenized with an Ultra Turrax (IKA Ultra-Turrax, Janke & Kunkel GmbH & Co KG, Staufen, Germany) for 60 s, and a further 10 ml TCA was added. The samples were stored on ice to prevent heating during the homogenization process. The homogenized samples were centrifuged for 15 min at 2000 rpm and 4°C. Then, the homogenate was filtered through a Whatman No. 4 filter, aliquoted, and stored at −80°C until further processing. For the TBA reaction, 100 μl of the thawed homogenates was transferred to reaction tubes. After adding 200 μl TCA (10%), samples were incubated on ice for 5 min and then centrifuged for 6.5 min at 13.200 rpm and 4°C. The supernatant was taken and diluted in Aqua Bidest (1 : 2.5). TBA reagent was added to the samples and then incubated at 100.5°C for 60 min. The samples were then cooled in a 4°C centrifuge at 8000 rpm for 2 min. Samples were transferred to microplates and quantified in a microplate reader at excitation/emission 515/553 nm.

4.3.3. Microbiological Analyses

Microbiological analyses were conducted to assess the initial contamination of the meat samples and to investigate the proliferation of typical spoilage organisms. The focus of the analyses was on total viable count (TVC) which was analyzed for every sample (n = 219). *Pseudomonas* spp., *Brochothrix thermosphacta*, and Enterobacteriaceae were analyzed for a subset of samples (n = 119). For microbial investigations, a standardized surface of meat tissue, with a size of $5\,\mathrm{cm}^2$, was extracted aseptically using a sterile punch and a scalpel. The samples were transferred to a sterile, filtered stomacher bag. The ninefold amount of saline peptone diluent (0.85% NaCl with 0.1% peptone Saline

tablets, Oxoid BR0053G, Cambridge, United Kingdom) was added with an accuracy of 0.1 g for the first dilution step. The samples were mixed with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60 s. Tenfold dilutions of the homogenate were prepared in saline peptone diluents.

The total viable count (TVC) was determined by the pour plate technique on Plate Count Agar (PCA, Merck, Darmstadt, Germany). The plates were incubated at 30°C for 72 h. *Pseudomonas* spp. (PSE) were detected by the spread plate technique on Pseudomonas agar with Cetrimide-Fucidin-cephalosporine selective supplement (CFC, Oxoid, Cambridge, United Kingdom). Plates were incubated at 25°C for 48 h. *Brochothrix thermosphacta* was determined by the drop-plate technique on SIN agar (Streptomycin Inosit Toluylene Red Agar, Oxoid Limited, Basingstoke, United Kingdom) and counted after incubation at 25°C for 48 h. Enterobacteriaceae were identified using the pour plate technique with overlay treatment on Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany). VRBD plates were incubated 24 h at 37°C.

4.3.4. Sensory Investigations

Sensory investigations were conducted by a trained sensory panel (four panelists) for a total of 221 filets. The analyses comprised the PD, assessment of WS, and the characterization of the freshness loss via the sensory index (SI). The PD was assessed via dichotomic response options. In detail, the panelist had to decide whether they would buy the product in a closed package or not. WS was graded via a three-point scoring system from 0 to 2 (0, no WS; 1, medium WS; 2, severe WS). For assessing freshness, poultry filets were evaluated based on a graded five-point scoring system with five meaning highest quality and one meaning spoiled. The evaluation was performed for the parameters color, odor, texture, meat juice color, and meat juice quantity for each sample. The sensory index (SI) was calculated as a weighted average with the following equation 4.2:

$$SI = \frac{2 \cdot O + 2 \cdot C + T + JC + JA}{7} \tag{4.2}$$

where SI is Sensory Index, O is odor, C is color, T is texture and JC is meat juice color and JA is meat juice amount.

According to the scheme, the product is spoiled when the SI reaches the level of 2.3. The SI was plotted as a function of time and fitted to a linear model. Thus, the shelf life of each sample was calculated following the procedure in (Kreyenschmidt, 2003).

4.3.5. Data Analysis and Statistics

Microbial data were $\log_{10}$ transformed and plotted as function of time. The data were fitted to a nonlinear model (Levenberg–Marquardt algorithm) using the software package OriginPro 8G (OriginLab Corporation, Northampton, MA, USA). To describe the microbial growth curve, the modified Gompertz model was used (Gibson et al., 1987):

$$N(t) = A + C \cdot e^{-e^{-B \cdot (t-M)}} \tag{4.3}$$

with N(t): microbial count log_{10} [cfu/g] at time t, A: initial bacterial count (lower asymptotic line), C: difference between upper asymptotic line of the growth curve (Nmax= maximum population level) and the lower asymptotic line, B: relative growth rate at time M [1/h], M: time at which maximum growth rate is obtained (reversal point), t: time [h].

When TVC reached a level of $\log_{10}$ 7.5 cfu/cm^2 the product was considered as spoiled. Microbial shelf life was calculated by transforming equation 4.3 and including the calculated model parameters.

Since criteria for normal distribution and homoscedasticity were not met by most of the parameters, nonparametric testing was selected for all statistical analyses. Differences between both production lines were analyzed with the Mann–Whitney U test using SPSS Statistics 23 (IBM Corporation 1989, 2013, New York, USA). Spearman's rank correlation and correlation plots were performed using the package corrplot and the software R (R Development Core Team, http://r-project.org). Multivariate testing was discarded due to the sample size at the single investigation points.

4.4. Results

The analysis of the nutritional value and muscle characteristics revealed significant differences between both production lines (Table 4.1). The content of intramuscular fat and water is significantly lower for the alternative filets. Besides, the content of protein is significantly higher compared to the conventional poultry meat. There was no significant difference for collagen. The level of L-lactic acid and D-glucose was significantly increased for the alternative filets. Both parameters significantly affected the pH-value (Figure 4.1). Higher amounts of L-lactic acid lowered the pH-value (k: -0.806, $p<0.001$, $n = 23$) and high amounts of D-glucose (k: -0.541, $p<0.001$, $n = 23$).

The mean pH-value of the filets was 6.25 for the alternative and 6.30 for the conventional production line at the beginning of storage (Table 4.2). The pH-value remained

Table 4.1.: Analysis of nutrients in the alternative and conventional production line, mean values, and standard deviations.

	Collagen [%]	Intramuscular Fat[%]	Protein [%]	Water [%]	N	L-Lactic acid	D-Glucose	N
Alternative	0.90	**1.01**	**23.68**	**74.25**	16	**0.952**	**0.056**	15
	±0.155	**±0.208**	**±0.534**	**±0.454**		**±0.0598**	**±0.0173**	
Conventional	0.85	**1.48**	**22.37**	**75.30**	16	**0.786**	**0.038**	8
	±0.179	**±0.473**	**±0.778**	**±0.562**		**±0.0173**	**± 0.0161**	

Bold parameters indicate differences between the production systems significant at the 0.05-level

stable during storage and showed an increase at 240 h. At the end of storage, the pH increased to 7.28 for alternative and 7.34 for conventional filets. The pH-values for the alternative group were lower at every investigation point, but the difference is only significant for the investigation after 72 h and 168 h.

The measurements for cooking loss and thawing loss showed a high variation between the groups. The mean cooking loss was slightly lower for alternative (14.13%) than for conventional filets (16.54%), but the difference was not significant (Table 4.2). At the beginning of storage, the thawing loss was 5.05% for alternative and 4.89% for conventional filets. For both production lines, thawing loss declined until the end of storage. Altogether, no significant difference in the water binding capacity was detected between either production line. Both parameters showed a significant negative correlation to the pH-value (cooking loss k:-0.616, $p<0.001$, n=22; thawing loss k:-0.599, $p<0.001$, n=22).

The color measurements of the filets revealed remarkable differences. The L*-value was lower at the alternative filets at most investigation points with significant differences after 120 h and 168 h of storage. For both production lines, the L*-value decreased significantly during storage with a mean ΔL* of 5.30 for alternative and ΔL* of 4.38 for the conventional production line. The L*-value was negatively correlated to pH (k:-0.614, $p<0.001$, n=196) and positively correlated to the thawing loss (k:0.762, $p<0.001$, n=43). The a*-values were significantly higher for the alternative group at all investigation points, meaning a higher amount of red color in comparison to the conventionally produced filets. The a*-values showed no clear development during storage for both investigation groups. The b*-values were significantly higher for the alternative filets. As for the a*-values, also the b*-values showed no clear trend during storage. Altogether, the color values showed, that the alternative filets display more yellowish filets with a darker color. In comparison, the conventional produced filets were more pale and showed a light pink color.

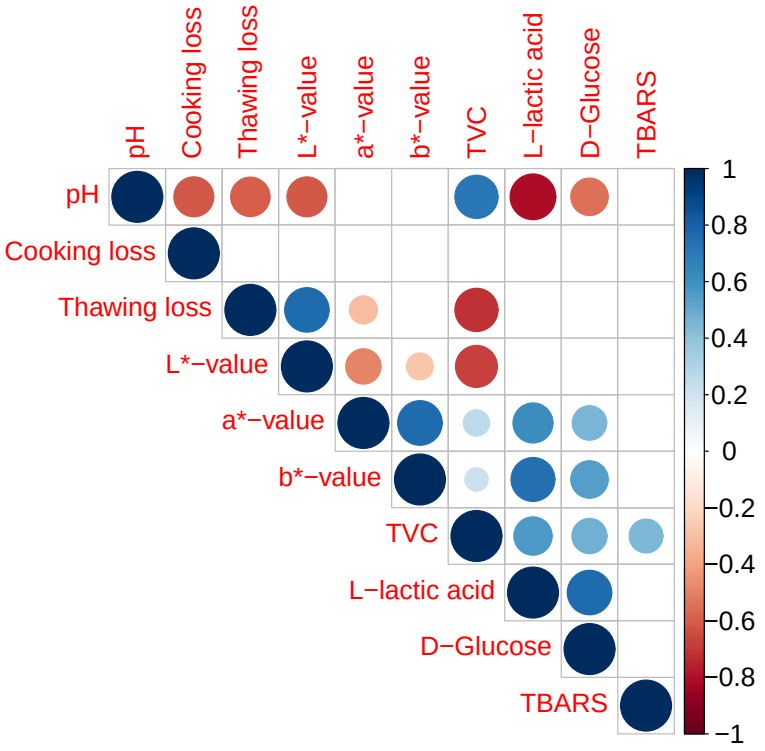

Figure 4.1.: Correlation between physicochemical parameters. Only correlations significant at the 0.05-level are displayed.

The investigation of TBARS revealed no significant differences between the groups. At the beginning of storage, mean TBARS were 0.120 mg MDA/kg meat (alternative) and 0.115 mg MDA/kg meat (conventional). TBARS showed an increase during storage, but standard deviations were very high and showed a broad overlap between investigation points and investigated groups (see Appendix Figure A.1, p.136). After 240 h storage, mean TBARS were 0.133 mg MDA/kg meat (alternative) and 0.144 mg MDA/kg meat (conventional). Regarding the overall storage time, TBARS was significantly correlated to TVC (k: 0.442, $p<0.001$, $n=131$). Additionally, TBARS was correlated significantly to all sensory parameters, especially odor (k: 0.499, $p<0.001$, $n=131$), color (k: 0.490), and the color of the meat juice (k: 0.487, $p<0.001$, $n=131$).

Regarding the microbial investigations, the initial TVC differed significantly and was higher for alternative filets (2.44 $\log_{10}$ cfu/cm^2) than for the conventional filets (2.10 $\log_{10}$ cfu/cm^2). During storage, this difference in initial contamination vanished with the proliferation of the microorganisms.

Table 4.2.: Mean values of investigated meat quality parameters during storage

	0 h	72 h	120 h	168 h	240 h	288 h
Weight [g]						
Alternative	277.44±36.16					
Conventional	300.74±28.19					
pH						
Alternative	6.25±0.11	**6.15±0.13**	6.24±0.10	**6.24±0.13**	6.95±0.49	7.28±0.35
Conventional	6.30±0.23	**6.30±0.18**	6.29±0.16	**6.35±0.13**	7.24±0.29	7.34±0.46
Cooking loss [%]						
Alternative	14.13±1.63					
Conventional	16.54±4.52					
Thawing loss [%]						
Alternative	5.05±1.83					2.29±0.79
Conventional	4.89±1.24					3.12±1.45
L*-value						
Alternative	57.82±1.95	56.41±1.97	**55.79±2.45**	**55.60±1.71**	55.59±1.44	51.21±2.49
Conventional	58.35±0.96	57.43±2.67	**57.80±2.36**	**57.55±2.35**	54.12±2.29	52.85±3.28
a*-value						
Alternative	**5.25±0.64**	**5.89±1.18**	**5.77±1.46**	**5.75±0.90**	**5.73±0.80**	**6.70±1.49**
Conventional	**4.07±0.54**	**3.87±0.53**	**4.15±0.98**	**3.87±0.79**	**4.15±1.21**	**4.41±1.21**
b*-value						
Alternative	**21.17±1.67**	**24.86±4.23**	**25.32±3.67**	**25.14±3.25**	**26.60±3.91**	**24.95±3.34**
Alternative	**16.45±1.55**	**13.29±0.81**	**14.93±4.64**	**14.16±1.16**	**18.07±2.22**	**17.57±2.37**
TBARS [mg MDA/kg]						
Alternative	0.120±0.018	0.115±0.013	0.121±0.011	0.132±0.017	0.152±0.019	0.133±0.028
Conventional	0.115±0.014	0.111±0.011	0.118±0.13	0.119±0.014	0.150±0.017	0.144±0.021

Bold parameters indicate differences between production systems significant at the 0.05-level

The initial counts of *Pseudomonas* spp. were below the detection limit 24 h after slaughter. Pseudomonads were growing dominant during storage and were on the same level as TVC. Thus, *Pseudomonas* spp. can be confirmed as Specific Spoilage Organism (SSO) (Figure 4.2) for both groups. TVC reached a maximum of 9.43 $\log_{10}$ cfu/cm^2 and 9.34 $\log_{10}$ cfu/cm^2, respectively. The maximum of *Pseudomonas* spp. was at 9.35 $\log_{10}$ cfu/cm^2 for alternative and 9.30 $\log_{10}$ cfu/cm^2 for conventional produced meat. No differences were observed for the progression of the growth curve of TVC as well as *Pseudomonas* spp. for both meat types. For both investigation groups, no significant differences of inital bacterial counts for *B. thermosphacta* and Enterobacteriaceae were detected, since bacteria were under the detection limit 24 h after slaughter. The growth of *B. thermosphacta* reaches a mean maximum of 6.72 $\log_{10}$ cfu/cm^2 (alternative) and 6.61 $\log_{10}$ cfu/cm^2 (conventional) after 288 h of storage. Enterobacteriaceae displayed mean maximum values of 5.86 $\log_{10}$ cfu/cm^2 and 6.04 $\log_{10}$ cfu/cm^2 was reached after 288h. Thus, the development of microbial growth was very similar for both investigation groups.

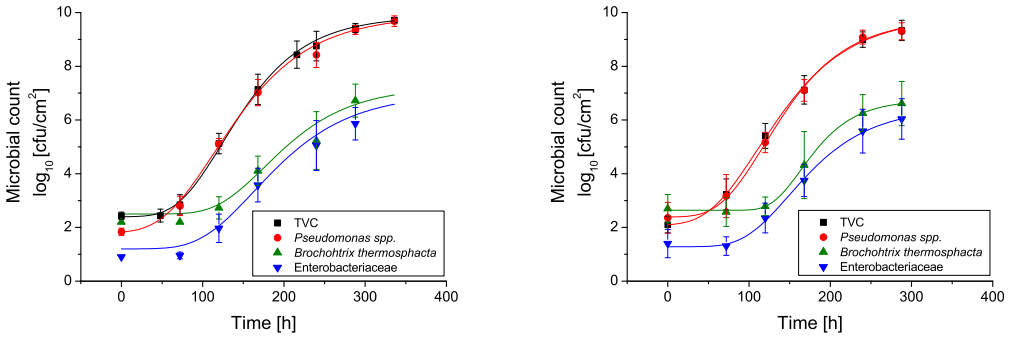

Figure 4.2.: Evolution of microbiological contamination on filets of the alternative(a) and conventional(b) production line

The shelf life also showed no differences between both investigation groups. The microbial spoilage level of 7.5 $\log_{10}$ cfu/cm^2 for TVC was reached by the alternative filets after 178 h and by the conventional produced filets after 175 h (Figure 4.3). The sensory shelf life was reached after 201 h (alternative) and 192 h (conventional), respectively. The alternative group achieved better scores at a few investigation points, mainly due to better evaluations for color or odor. However, these discrepancies did not lead to a significant difference in shelf life.

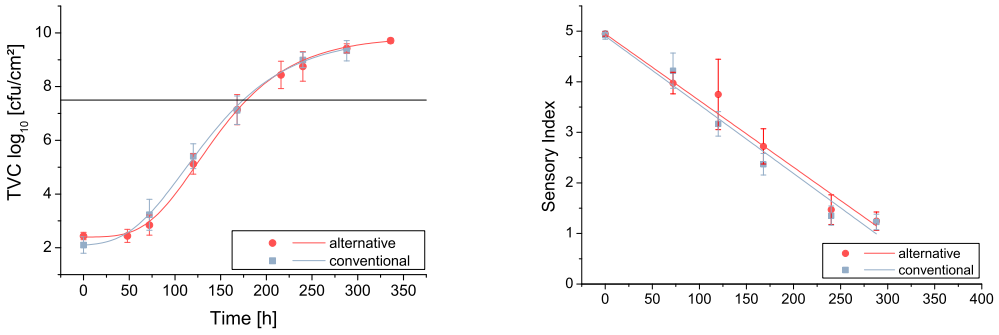

Figure 4.3.: Shelf life of alternative and conventional produced poultry filets determined by microbial contamination(a) and sensory index(b)

Severe WS was observed most in the conventional production line at every investigation point, but the difference is not significant. There was no clear tendency or pattern, indicating that the categories “no WS” or “medium WS” developed differently in either of the investigation group (Figure 4.4). Additionally, no effect of storage on the display of WS could be observed. Due to the strong discoloration of the filets caused by spoilage,

the incidence of WS is only displayed until 168 h of storage. WS showed a significant negative correlation to the b*-value (k:-0.426, p=0.048, n=22) at the first investigation point, meaning that more yellowish filets showed a less pronounced WS. Regarding the overall storage time, WS was significantly correlated to the pH (k: 0.377, p<0.001) and significantly affected the PD negatively, but the correlation was low (k: -0.271, p<0.001, n=221). Moreover, WS was not correlated to filet weight or any other physicochemical parameters.

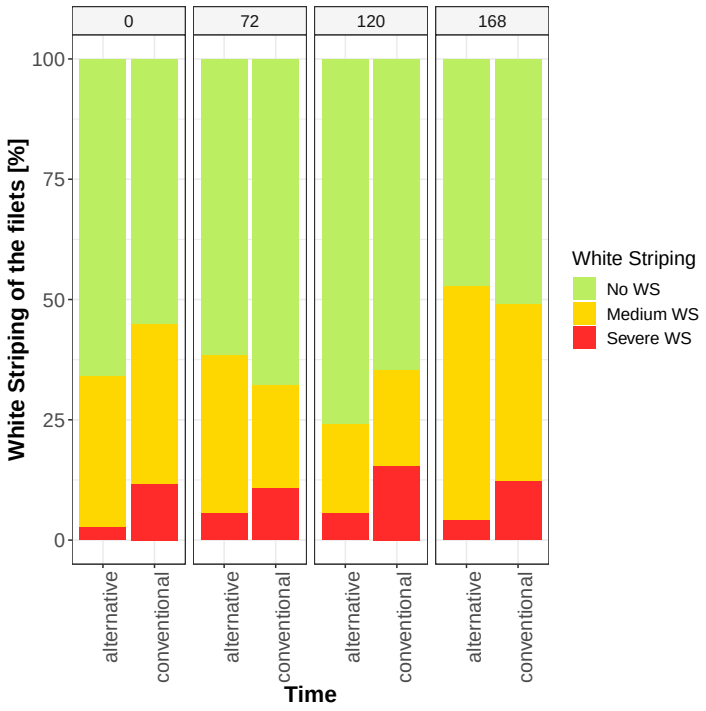

Figure 4.4.: Occurrence and severity of WS for both production lines during storage

The PD was higher for alternative filets at every investigation point (Figure 4.5). This difference is significant for the first investigation point. The PD declined during storage, as the meat showed a loss of freshness. A high rejection rate of the samples was obvious after 120 h and less than 10% were accepted after 168 h of storage. All filets were rejected by the panel after 240 h, when the filets were spoiled. The PD was influenced significantly by the other parameters assessed by the sensory panel, especially color (k: 0.910, p<0.001, n = 221) and WS. All other sensory parameters were also significantly correlated to the PD with correlation coefficients between 0.8 and 0.9. Additionally, PD was correlated to the L*-value (k: 0.461, p<0.001, n = 196) with a higher rejection rate for darker filets.

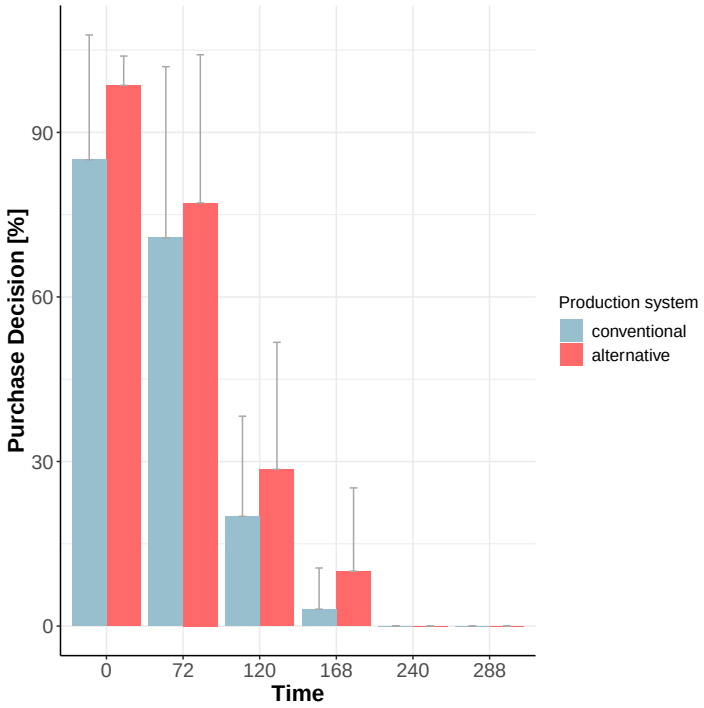

Figure 4.5.: Purchase decision evolution during storage.

4.5. Discussion

The results of this study showed that different production systems can have a significant influence on biochemical composition, nutritional value, and physicochemical characteristics of poultry meat. Generally, the nutritional values and muscle characteristics of the investigated poultry filets were comparable to former studies (Dave and Ghaly, 2011; Meluzzi et al., 2008; Castellini et al., 2002). Filets of the alternative line had significantly higher protein and lower water and intramuscular fat content in comparison to the conventional production line. Different dietary strategies are known for their ability to modify the nutritional profile of poultry meat (Bou et al., 2009). Maize-based diets provide an easily accessible source of energy leading to a higher protein conversion in comparison to wheat-based feed (Jamroz et al., 2005). Additionally, the race and the opportunity for a regular exercise can lower the fat and increase the protein content of poultry meat (Fanatico et al., 2009). However, detailed analyses focusing on both investigated breeds are lacking. A higher motor activity favors myogenesis over lipogenesis as stated by Castellini et al. (2002). Thus, a higher meat quality of the filets was observed in the alternative production line with a maize-based diet, lower stocking

density, and enhanced motivation for physical activity by offering toys. The amount of L-lactic acid and D-glucose in the muscle was comparable to the results of Bruckner et al. (2012a). The higher values of L-lactic acid and D-glucose can be explained by a higher glycolytic potential in the muscle of the alternative production line (Monin et al., 1987). The glycolytic potential of the muscle ante mortem has been related to the breeding and fattening of the animals, stress, exercise levels, or age (Debut et al., 2003; Berri et al., 2008; Berri, 2000; Gill, 1983; Nychas et al., 1988). Additionally, the selection for growth rate and age influences the glycolytic potential and the pH decline postmortem (Wang et al., 2013; Berri et al., 2001). The lower pH-values observed for the alternative production line can be explained by the close relationship between L-lactic acid and pH-value (Bruckner et al., 2012a). Thus, the production system and choice of a specific slower growing race showed implicit consequences for technological traits of the product. For fresh poultry meat, the pH-value ranges between 5.8 and 6.2 (Bruckner et al., 2012a; Debut et al., 2003; Berri et al., 2001; Garcia et al., 2010). According to these studies, pH-values observed in this investigation were comparatively high. However, higher pH-values have been reported before for the Ross line and other modern poultry lines selected for fast growth and early slaughter age (Wang et al., 2009; Berri et al., 2001; Glamoclija et al., 2015). This is in agreement with the findings of this study in which the fast growing Ross 308 had higher pH-values than the slower growing line Ranger Classic. At the end of storage, the pH-value shows a significant decrease which is typical for high bacterial cell counts. This is caused by the accumulation of ammonia as a result of amino acid degradation when glucose decreased to insufficient levels (Gill, 1983). The pH-value was closely related to the cooking loss, thawing loss, and color values of the poultry filets, which is in accordance with former studies (Castellini et al., 2002; Fanatico et al., 2007; Petracci et al., 2004). Even though the dietary composition and breed were reported to have an impact on water holding capacity of the meat (Berri et al., 2008; Albrecht et al., 2017; Downs et al., 2000; Young et al., 2004), no differences in cooking and thawing loss could be detected between the production lines in this study.

The L*-values showed no significant difference between groups but were higher than the optimal range for poultry reported in former studies (Petracci et al., 2004; Woelfel et al., 2002; Barbut, 1997; Allen et al., 1998). Since cooking and thawing loss were in a normal range, a pale soft and exudative- (PSE-) like condition was not observed according to the criteria defined in former studies (Woelfel et al., 2002; Barbut, 1997). Besides, a high variation in the L*-value of fresh poultry meat has been reported before and was explained by different pre-slaughter and processing conditions, resulting in

varying L*-values (Petracci et al., 2004). The decrease of L*-values during storage for both investigation groups can be explained by the biochemical degradation of myoglobin and is typical for the spoilage of meat (Faustman and Cassens, 1990). Differences in the a*- and b*-values between groups reflected the intense and more yellowish color of the filets of the alternative production. This effect was caused by the maize-based diet and higher amount of carotenoids (Lyon et al., 2004). During storage, a*-values and b*-values of the alternative line increased, while only b*-values of the conventional line showed a slight increase. Thus, the alternatively produced filets show no typical discoloration process during spoilage, characterized by a fading of the pink color typical for fresh meat. The filets rather displayed a change to a darker and more orange color.

The investigation of TBARS showed no significant difference between both production lines, even though an influence of animal diet on lipid oxidation has been reported before (Karre et al., 2013). During storage, TBARS values showed a significant increase which is in accordance with former studies (Sujiwo et al., 2018; Luna et al., 2010; Aksu et al., 2005). Thus, the differences in physicochemical properties measured between both groups did not result in a varying spoilage process. Significantly higher levels of glucose, which is a key substrate for microorganisms (Nychas et al., 2008), could indicate an accelerated microbial growth on filets of the alternative group, but this was not observed. Since animals of both production lines were slaughtered and processed in the same production facility on the same day, the amount and diversity of contaminating and proliferating microorganisms showed no relevant difference. For both groups, the initial TVC contamination was low in comparison to other studies conducted under industrial slaughter conditions (Bruckner et al., 2012a; Vasconcelos et al., 2014; Sahar and Dufour, 2014; Raab et al., 2008). The abandonment of antibiotics showed no impact on microbial contamination or growth in this study. For both groups, the microbial shelf life was in accordance with the sensory shelf life and is in the normal range for fresh, aerobically packaged poultry (Albrecht et al., 2017). Shorter shelf lives for similar products were reported as well but could be related to higher initial microbial contamination (Bruckner et al., 2012a; Raab et al., 2008; Bruckner et al., 2012b). Both production systems resulted in high quality poultry products with no implications for the spoilage process and shelf life. Since the usage of antibiotics in meat production has a high impact on environment, the increase of antibiotic resistances, and human health (Silbergeld et al., 2008; Singer and Hofacre, 2006), antibiotics-free systems reveal important opportunities towards a more sustainable poultry production. According to the results of this study, the realization of an alternative production system without antibiotics is possible without

impacts on meat quality and shelf life. The decelerated growth of the animals and gentle fattening had no impact on the incidence of WS. Even though the conventional group displayed the highest occurrence of severe WS, no significant difference could be detected between the investigation groups. WS was significantly correlated to growth rate, genotype, slaughter age, and filet weight in former studies (Petracci et al., 2015; Kuttappan et al., 2016, 2012a), but no significant correlation between the filet weight and WS was observed in this study. In contrast to former studies reporting an effect of WS on the water binding capacity (Sanchez Brambila et al., 2016; Mudalal et al., 2015; Petracci et al., 2013), no effect of WS on drip or thawing loss was observed here, also stated by (Kuttappan et al., 2013). As a result of WS incidence, the PD was affected. A low consumer acceptance for filets displaying WS was also observed before (Kuttappan et al., 2012a). The PD was mainly dominated by the color of the filets. A tendency for a preference for the alternative filets was observed, but the difference was only significant at the first investigation point.

4.6. Conclusion

The alternative line encompasses the opportunity towards a more sustainable poultry production due to an extensive husbandry system without antibiotics, a slower growth, and enhanced animal welfare. This investigation revealed a significant benefit for the biochemical composition and nutritional value of alternatively produced poultry meat. The poultry filets of both production lines showed an overall high quality, and no effect of the production system on the development of quality parameters and shelf life could be detected. The abandonment of antibiotics in the alternative line had no impact on the microbial quality, safety, or shelf life of the product. The decelerated growth of the animals did not lead to a significant improvement for the incidence of WS in comparison to conventional production. The PD was negatively influenced by WS and higher for breast filets from the alternative production system. A trial repetition to confirm the findings with a higher sample size is desired.

4.7. Acknowledgments

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5. Fattening Process and Animal Health in Relation to the Quality and Shelf Life of Pork Meat

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To be submitted

5.1. Abstract

The influence of pig performance, animal welfare and health in relation to typical meat quality paramters and shelf life was investigated. During this trial, 84 pigs were raised and fattened in an experimental farm. The fattening process was accompanied by weight measurements every three weeks, estimation of the food conversion rate as well as characterization of the health status by a veterinarian. At a final weight of 108 kg, the animals were slaughtered in an industrial slaughterhouse. The investigation of meat quality was conducted 24 h postmortem and comprised the nutritional value (protein, collagen, water, intramuscular fat), meat color, pH-value and electric conductivity of filet slices of the *M. longissimus dorsi*. Additionally, drip loss, cooking loss, thawing loss and shear force were investigated. The filets were stored aerobically at 4°C and microbial as well as sensory investigations were conducted to characterize the spoilage process of the meat. The quality parameters, microbiology as well as shelf life showed a broad variation. The shelf life of the filets was significantly influenced by the weaning age of the animals, the water content in the meat as well as the pH decline postmortem and electric conductivity. Further influencing factors were sex and breed. Even though individual animals with clinical findings also showed a tendency towards a shortened microbial shelf life, this difference was not significant. Besides, antibiotic medication showed no significant effect on shelf life.

5.2. Introduction

Pork meat production is a succesful industry sector showing a remarkable growth potential on a global scale (Godfray et al., 2010; Henchion et al., 2014; Sans and Combris, 2015). To meet the increasing consumer demands for high quality animal based proteins, the meat industry underwent a striking change towards higher efficiency and animal turnover rates in the last decades (Trienekens et al., 2009; Maples et al., 2017). Vertical integration, optimized breeds as well as diets highly adjusted to the animals needs led to a faster production of goods affordable to the consumer (Dunshea et al., 2005; Reimer, 2006; Sosnicki and Newman, 2010). With the intensification of meat production several consumer concerns arose. Ethical attributes such as animal health and welfare as well as sustainability are gaining importance (Verbeke and Viaene, 2000). Growing concerns about public health, food safety or environmental risks were caused by various food scandals as well as reports about water pollution and harmful residues (Walker

et al., 2005). Thus, the meat industry today faces the challenge of efficiently producing high amounts of affordable products, under the consideration of sustainability, animal health and welfare problems at the same time (Dawkins, 2016). As a result, a variety of pork production systems with different levels of intensification emerged across Europe (Verbeke and Viaene, 2000). But the impact of ethical questions about animal health and welfare are not limited to consumer concerns. The handling of the animals is known to have a significant effect on the technological traits and processing characteristics of the product (Velarde et al., 2015). Husbandry and pre-slaughter conditions encompass a large stress potential for the animals with implications for pork meat quality as well (Schwartzkopf-Genswein et al., 2012; Faucitano, 2018). Hence, animal welfare and health are supposed to be intrinsically tied to meat quality. Beyond that, pork meat quality is characterized by complex properties and determined by different interacting factors. The genotype, especially the halothane gene and the RN- gene, has a direct impact on technological meat quality by influencing the development of pale, soft and exudative (PSE) meat or the pH drop postmortem (Monin et al., 1987; Leach et al., 1996; Brewer et al., 2002; Rosenvold and Andersen, 2003a). Carcass characteristics and meat quality are also influenced by a variety of housing and feeding conditions of the pigs. The diet determines the leanness of the meat and offers the opportunity to manipulate the muscle glycogen stores with effects on ultimate pH and meat color (Pettigrew and Esnaola, 2001; Rosenvold and Andersen, 2003b). These properties are also influenced by rearing conditions or free-range access (Gentry et al., 2002; Lambooij et al., 2004; Millet et al., 2004). Fattening and housing of the pigs is related to the performance of the animals, carcass characteristics and meat quality as well (Millet et al., 2004). The pH and physicochemical meat composition have a striking influence of microbiological growth and spoilage of fresh pork meat (Borch et al., 1996; Holmer et al., 2009). But during the investigation of different husbandry conditions, the development of meat quality and shelf life is often not considered. Thus, when exploring extensive production systems, research should not be limited to the performance, carcass traits, stress and health of the animals. The storage ability of the meat product and shelf life should also be taken into account.

Thus, the aim of the study was the investigation of husbandry conditions, including weaning, performance parameters, sex and breed on physicochemical and microbial properties of fresh pork meat. The influence of these parameters, in combination with health status and animal welfare, on the quality and shelf life of fresh pork was tested.

5.3. Material and Methods

5.4. Study Design

Housing and fattening of 84 pigs was conducted in the experimental farm of the University of Bonn in nine batches. Breeding boars were German Piétrain and sows were German Landrace (DL) as well as hybrid sows (DExDL; German Noble Race x German Landrace). The mean birth weight of the pigs was 1.71 kg (±0.35). Piglets were separated from sows at the age of 29 days (±4) and at 9 kg of weight. The sex ratio was well balanced (47 female, 37 male) and male pigs were castrated during the first week of life. Fattening started at the age of 70 d and a weight of 26.7 kg (±4.26). All pigs were raised under the same feeding conditions. Age and weight at the beginning of fattening and slaughtering was documented. During the fattening process, body weight was measured every three weeks to determine performance of the animals. Feed intake was recorded each day for each pen. Average daily gain (ADG, g/day) and feed conversion ratio (FCR, kg/kg) were calculated for the whole period of fattening: from the beginning of fattening until slaughter, and the period from 30 to 105 kg body weight, penwise.

The pigs were slaughtered and deboned in an industrial slaughterhouse in eight batches. The animals were slaughtered at a weight between 105 and 110 kg and a mean age of 175 d. The *M. longissimus dorsi* was taken as meat sample and transported under temperature-controlled conditions to the laboratory of the University of Bonn. The samples arrived in the laboratory 24 h after slaughtering. Meat samples were taken from the *M. longissimus dorsi* by cutting five slices (2 cm thick) under sterile conditions. The filets were packed aerobically and stored at 4°C in a high precision low temperature incubator (SANYO model MIR 153, Sanyo Electric Co., Ora-11 Gun, Gumma, Japan). The laboratory investigations started directly after arrival at the laboratory. The nutritional and meat quality analyses were conducted 24 h postmortem. For assessing microbial and sensory shelf life, the analyses were conducted in a time row of five measurements from the beginning (24 h postmortem) until the end of storage.

5.4.1. Meat Quality Analysis

The meat quality analysis comprised nutritional composition and physicochemical parameters and were conducted according to the description at Klauke et al. (2013). The water, collagen, protein and intramuscular fat (IMF) content of the meat samples were measured using near-infrared spectroscopy (NIRS). Slices of meat were chopped with

a food processor (Tefal La Mulinette, Groupe SEB Deutschland GmbH, Offenbach am Main, Germany). The meat paste was transferred to a measurement dish, then placed in a spectrometer (NIRS DS2500, Foss, Rellingen, Germany) and analyzed automatically.

The pH-value, color and electrical conductivity (EC) were measured on the filet surface with devices developed by the engineering office R. Matthäus (pH-Star, OPTO-Star, LF-Star by Ingenieurbüro R. Matthäus, Nobitz, Germany). Drip loss measurements were conducted using the bag method (48 h, 4°C). The thawing loss was assessed by freezing at -20°C and then thawing. The cooking loss was determined by shrink-wrapping the sample and heating at 75°C for 50 min. The samples were then cooled in a water bath at 15-20°C for 40 min. For calculating the drip loss, thawing loss and cooking loss, samples were weighed before and after treatment. The calculation was conducted using the following equation (5.1):

$$W_L = \frac{m_1 - m_2}{m_1} \cdot 100\% \tag{5.1}$$

where W_L is water loss [%], m_1 is mass before treatment, and m_2 is mass after treatment.

After cooking, the samples the tenderness was assessed by measuring Warner-Bratzler shear force (WBS) with an Instron apparatus (Instron LTd., UK) provided with a WBS tool.

5.4.2. Microbiological Analysis

For assessing total viable count (TVC), a representative meat sample of 25 g was taken with a cork borer and filled into a filtered stomacher-bag (Interscience, Saint Nom la Bretèche, FR). Saline peptone diluent (0.85% NaCl with 0.1% peptone; Oxoid, Basingstoke, United Kingdom) was added until a final weight of 250 g was reached. The sample was homogenized for 60 s with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany). A 10-fold dilution series was created using saline peptone diluents. Appropriate dilutions were transferred to plate count agar (PCA, Oxoid, Basingstoke, United Kingdom) and incubated at 30°C for 72 h.

5.4.3. Sensory Analysis

Sensory quality of each sample was evaluated by a trained sensory panel. Odor, texture and color were assessed following a 3-point scoring system where 3 = very good and 1 = unacceptable. A weighted sensory index (SI) was calculated using the following equation

(Kreyenschmidt, 2003):

$$SI = \frac{2 \cdot O + 2 \cdot C + T}{5} \tag{5.2}$$

where SI is Sensory Index, O is odor, C is color, T is texture and JC is meat juice color and JA is meat juice amount.

For assessing the shelf life of the meat, sensory acceptance was described as a function of time. Samples were considered "spoiled", when the SI reached 1.8 or lower.

5.4.4. Statistical Analysis

Microbial growth was fitted as a function of time using nonlinear regression (Levenberg-Marquardt algorithm). The modified Gompertz model was used to describe the logistic growth curve (Gibson et al., 1987):

$$N(t) = A + C \cdot e^{-e^{-B \cdot (t-M)}} \tag{5.3}$$

with N(t): microbial count [log_{10} cfu/g] at time t, A: initial bacterial count (lower asymptotic line), C: difference between upper asymptotic line of the growth curve (Nmax= maximum population level) and the lower asymptotic line, B: relative growth rate at time M [1/h], M: time at which maximum growth rate is obtained (reversal point), t: time [h].

The end of shelf life was achieved when total viable counts reached 7.0 $\log_{10}$ cfu/g. For statistical analyses, data were tested for normality and homoscedasticity using a K-S-test. Since data did not meet the requirements for parametric testing, non-parametric tests were used. Differences between groups, e.g. sex and breed, were tested pairwise using the Mann-Whitney-U test. A correlation analysis was performed with a Spearman's Rank correlation. A generalized linear model (GLM) was computed to identify main influence factors on the shelf life of filets. Response surface modeling (RSM) was conducted to explore and display the influence of the identified factors on the shelf life. Data analysis was conducted using SPSS 24 (IBM SPSS Statistics, USA), Origin Pro 8G (OriginLab Corporation, Northampton, MA) and R (R development Core Team, https://www.r-project.org/).

5.5. Results

An overview over performance parameters, meat quality and microbial parameters 24 h postmortem is displayed in Table 5.1. The birth weight of the piglets was between 0.9 kg and 2.5 kg with a mean weight of 1.71 kg.

Table 5.1.: Performance, quality and freshness parameters of the pigs 24 h postmortem

	N	Min	Max	Mean	SD	CV
Birth weight [kg]	84	0.9	2.5	1.71	0.35	0.21
Start weight [kg]	84	19	37	26.39	4.32	0.16
Slaughter weight [kg]	84	94	119	108.71	3.67	0.03
Weaning age [d]	84	25	36	29.14	3.85	0.13
Starting age [d]	84	65	75	70.01	4.24	0.06
Slaughter age [d]	84	143	223	175.42	13.16	0.08
Days in FU	84	78	148	105.40	13.66	0.13
FCR	84	2.26	3.36	2.59	0.17	0.07
ADG [g/d]	84	591	1087	817.25	102.29	0.13
IMF [%]	84	0.84	5.58	1.64	0.61	0.37
H_2O [%]	84	70.91	74.8	73.80	0.59	0.01
Protein [%]	84	23.51	25.53	24.33	0.44	0.02
Collagen [%]	84	1.16	1.59	1.34	0.09	0.07
Opto	84	58	83	67.26	6.69	0.10
pH_{24}	84	5.3	5.6	5.40	0.07	0.01
EC_{24}	84	2.2	4.1	2.87	0.45	0.16
Drip loss [%]	84	0.6	4.7	2.12	0.95	0.45
Cooking loss [%]	84	19.7	27.4	23.56	1.74	0.07
Thawing loss [%]	84	4.6	13.1	8.38	1.80	0.22
Shear force [N]	84	21.2	59.4	35.28	7.59	0.22
Microbial SL [h]	59	146.41	423.51	234.76	53.41	0.23
Sensory SL [h]	84	194.00	410.00	255.76	43.39	0.17
Y0	59	0.05	2.85	1.18	0.49	0.42
tlag	59	2.66	147.93	71.30	30.50	0.43
Nmax	59	7.06	11.61	8.51	1.08	0.13

FU - fattening unit, IMF - intramuscular fat, EC - electric conductivity, SL - shelf life

Piglets of the German Landrace x Piétrain breed (n=58) had a significantly lower birth weight than the Hybrid x Piétrain breed (n=26). Beyond that, the breed had no influence on any parameter during fattening. There was no significant difference in the birth weight, or other weight parameters, between male or female pigs. The mean FCR was 2.59 ($\pm$0.17) and mean ADG was 817.25 g/d ($\pm$102.29). Female pigs showed a significantly lower ADG ($p<0.001$), stayed longer in the fattening unit ($p<0.003$) and had a higher slaughter age ($p<0.001$). Beyond these differences, no significant differences between sexes were detected for the weaning and fattening process. No differences in performance parameters were detected between healthy animals and animals with clinical findings at the abattoir. Additionally, no influence of antibiotic medication on performance parameters was detected.

The meat samples showed a high initial quality upon arrival at the laboratory. The

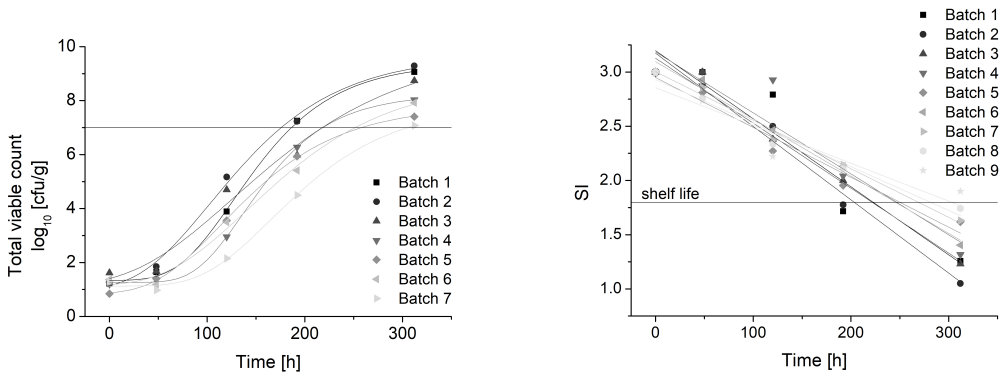

Figure 5.1.: Microbial (left) and sensory (right) shelf life of pork filets

mean pH-value ranged between 5.3 and 5.6, the mean value was 5.4 (±0.07). The drip loss ranged between 0.6% and 4.7% with a mean value of 2.12% (±0.95). Except for electric conductivity, there were no significant differences for meat quality parameters between both breeds. The EC_{24} was lower for the German Landrace x Piétrain breed in comparison to the Hybrid x Piétrain breed. Female pigs showed a significantly lower IMF ($p<0.001$) and higher water content ($p<0.001$) in the filets. The mean cooking loss was 23.56% (± 1.74) whereas female pigs showed a significant higher cooking loss than male pigs ($p<0.003$). Additionally, the shear force was significantly lower for male pigs ($p<0.032$). Beyond that, no significant differences in meat quality were detected between sexes. An influence of clinical findings or antibiotic medication on meat quality parameters could not be detected.

Microbial investigations revealed a low microbial contamination at the beginning of storage. The initial total viable count was between 0.70 and 2.74 $\log_{10}$ cfu/g with a mean value of 1.23 (±0.47). There was no significant difference in microbial parameters detected for the breed or the sex. The samples showed a normal spoilage process with a mean microbial shelf life of 235 h (±53.41). The growth of TVC showed variation among the different charges and is displayed in Figure 5.1.

The maximum bacterial counts ranged between 7.06 and 11.61 $\log_{10}$ cfu/g with a mean of 8.51 $\log_{10}$ cfu/g. The mean lag time was 30.50 h. The sensory investigations revealed a high initial quality and normal spoilage process with no significant differences between breeds or sexes. Mean sensory shelf life was 255 h (±43.39). No significant differences for microbial and sensory freshness parameters or shelf life was found between sexes or breed. Additionally, antibiotic medication showed no influence on freshness parameters. Clinical evidence was found for 22.6% of the pigs with 8.3% chronic pneumonia, 8.3%

salmonella, 3.6% pericarditis and pleurisy and 2.4% parasitic liver. For animals with clinical findings, a tendency towards a shorter microbial shelf life was observed (Figure 5.2).

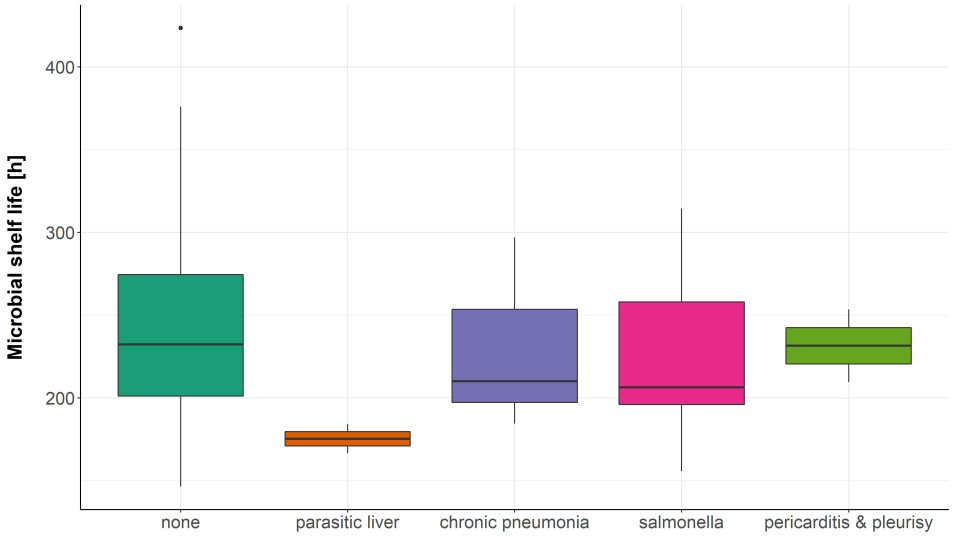

Figure 5.2.: Microbial shelf life for different diagnostic findings at the slaughterhouse

The mean microbial shelf life was 219.67 h (± 49.67) for sick animals and 240.66 h (±55.44) for healthy animals. This effect was less pronounced for the sensory shelf life with 245.58 h (±38.44) for sick and 258.74 h (±44.56) for healthy animals. The differences in shelf life were not significant (MSL: p=0.185, SSL: 0.219). The Spearman's Rank correlation revealed several correlations between breeding, fattening, meat quality and freshness parameters (Table 5.2). Higher birth weights of the animals were correlated to higher cooking loss and shear force of the filets. Lower body weights at entering the fattening process led to a longer microbial as well as sensory shelf life. Thus, animals with a start weight below 25 kg at the beginning of fattening showed longer shelf life times for the end products than animals entering the fattening process with 30 kg and above. The IMF was higher for animals with lower birth as well as weaning weights and increased with higher ADG. The content of IMF showed a direct effect on the cooking loss and shear force of the meat.

Table 5.2.: Spearman's Rank correlation coefficients of housing parameters, meat quality and shelf life

	Birth weight	Wean age	Wean weight	Start age	Start weight	Slau age	Days in FU	ADG	FCR	Opto	pH_{24}	EC_{24}	IMF	H_2O	Prot	Coll	Drip loss	Thaw loss	Cook loss	Shear force	MSL
Wean day																					
Wean weight	**.535**	.239																			
Start day	**-.278**	**.438**	-.240																		
Start weight				**.490**																	
Slau age					**-.299**																
Days in FU				**-.327**	**-.527**	**.903**															
ADG						**-.809**	**-.796**														
FCR		**.465**				**.408**	**.352**	**-.378**													
Opto		**.317**		**.321**																	
pH_{24}										**.555**											
EC_{24}																					
IMF	**-.444**		**-.315**				-.236	**.365**		-.265		-.217									
H_2O	**.337**	-.229			.225						**.326**		**-.461**								
Prot				**.352**	.241									-.220							
Coll										-.252					-.275						
Drip loss								-.216			**-.339**	**.319**									
Thaw loss		**.312**	.277	-.225																	
Cook loss	**.400**		**.405**						**-.294**				**-.500**	**.465**							
Shear force	**.281**	**.316**	.256			.278	.252			**.457**	**.405**		**-.288**						.237		
MSL		**.435**			**-.326**		.312							**-.474**		.283		**.390**			
SSL					-.276	**.309**	**.331**		**.300**												**.536**

Only correlations significant at the 0.05-level are displayed. Bold values mark correlations significant at the 0.01-level.

FU - fattening unit, ADG - average daily weight gain, FCR - feed conversion rate, IMF - intramuscular fat, Prot - protein content, Coll - collagen content, MSL - microbial shelf life, SSL - sensory shelf life

With more IMF in the meat, the filets showed a lower water content, cooking loss and also shear force. A higher water content in the filets led to higher cooking loss and reduced microbial shelf life. The pH-value was negatively correlated to the drip loss and positively correlated to the water content. Additionally, the meat color and shear force were influenced by the pH-value.

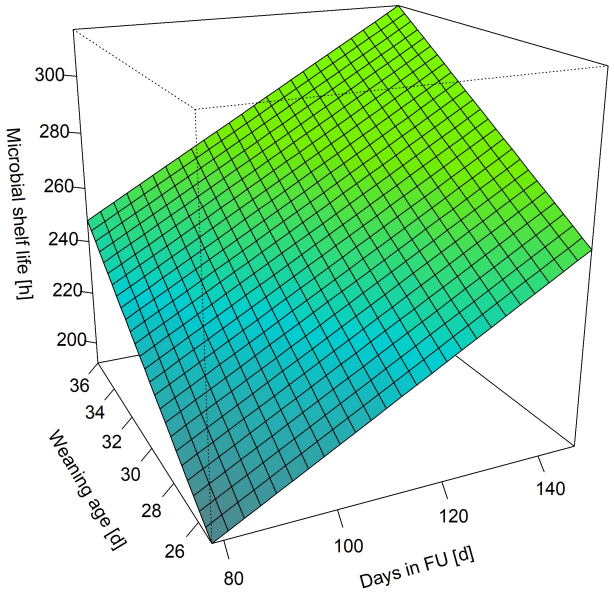

Figure 5.3.: Twofold dependency of microbial shelf life from weaning day and days in fattening unit (FU)

The performance parameters showing the highest correlations to shelf life were 'Weaning age', 'Starting weight' and 'Days in the FU'. Since 'Starting weight' and 'Days in FU' are closely related, the parameters 'Weaning age' and 'Days in FU' were selected for further modeling. Response surface modeling was conducted to describe the twofold dependency of microbial shelf life from these parameters (Figure 5.3). The figure illustrates that animals weaned at a younger age and who had a shorter abidance in the fattening unit developed meat showing the most rapid microbial spoilage process. A later weaning and more time during the fattening process led to a longer microbial shelf life. Generalized linear modeling was conducted to identify predictors for microbial shelf life. All parameters on performance and quality were included as well as sex, breed, health status and antibiotic medication as fixed factors. The analysis revealed that sex

and breed had a significant influence on shelf life, but not the health status or antibiotic medication (see Table A.3, page 137). Weaning age was identified as highly significant performance parameter to predict microbial shelf life. For the quality parameters, the content of water, EC_{24} and pH decline after slaughter were significant model effects.

5.6. Discussion

The early stages of pig production showed a significant influence on meat quality parameters and shelf life in this study. Typical influence factors reported for pork meat quality are, among others, breed, diet, stress or rearing conditions (Pettigrew and Esnaola, 2001; Rosenvold and Andersen, 2003a; Faucitano et al., 2010). Besides, birth weight and early postnatal growth of the piglets effect fiber and muscle structure with direct implications for meat quality (Gondret et al., 2005, 2006; Beaulieu et al., 2010). In this study, the birth weight of the piglet was related to the content of IMF and water in the meat, which is in accordance with former studies (Gondret et al., 2006; Rehfeldt et al., 2008). Higher water content in the pork filets significantly reduced shelf life in this investigation which can be explained by an accelerated growth of microorganisms (Borch et al., 1996; Dave and Ghaly, 2011). Additionally, the weaning age had a significant effect on typical meat quality parameters. Ko et al. (2015) reported that weaning age and weight influenced pork carcass characteristics as well as meat quality traits and was supposed to be as important as performance parameters during the fattening process. This is supported by the findings of this investigation where the weaning age was an important predictor for microbial shelf life. Especially in combination with duration of the fattening process, the effect was considerable. Pigs weaned at a young age and a short time in the fattening unit, which is equivalent to a fast growth, showed the lowest shelf life. A young weaning age could be balanced by a longer fattening process and slower growth at the finishing stage. The growth of the animals determines muscle fiber types, metabolic traits as well as the glycolytic potential of the muscle (Karlsson et al., 1993; Klont et al., 1998). The velocity of growth is often determined by breed or husbandry conditions, with implications for the heterogeneity in glycogen depletion postmortem. The extent of pH decline is closely related to meat quality parameters and shelf life (Rosenvold and Andersen, 2003a; Barbut et al., 2008; Faucitano et al., 2010), as it was also observed in this study. Thus, a more extensive production is supposed to improve animal welfare with benefits for meat quality as well as shelf life. In general, the pH-values and other meat quality traits in this study were in a normal range for fresh pork meat (Huff-Lonergan et al.,

2002; Faucitano et al., 2010; Bruckner et al., 2012). The shelf life of the investigated filets was longer than reported before which can be explained by the low initial contamination on the filets (Bruckner et al., 2012). There was a high variation in shelf life among the slaughter batches. This can can not be explained by initial microbial counts which are varying only in a small range. Since the animals with the shortest abidance in the fattening unit and the most rapid growth were slaughtered first, the influence of the performance parameters was also reflected by a correlation between batch number and microbial shelf life. This effect was also visible in the sensory evaluation of the spoilage process. Besides the mentioned effects of performance and quality parameters on shelf life, sex and breed were significant predictors. Their main influence on shelf life can be explained by variation in the IMF and H_2O content of the meat. The effect of breed and sex on lean meat and water content is in agreement with former studies (Latorre et al., 2003; Piao et al., 2004; Jeleníková et al., 2008), but, up to our knowledge, an impact on the shelf life of the product has not been reported yet. Animals with clinical findings showed a tendency to develop filets with a shorter shelf life. However, the health status of the animals showed no significant influence on meat quality or shelf life. Since the animals with clinical findings comprised only a small group, a more detailed investigation with a higher sample size is required to investigate these relationships. Additionally, antibiotic medication had no influence on typical quality, freshness parameters or shelf life of the meat. In general, this investigation confirmed that typical meat quality parameters are determined by sex, breed and performance parameters as several studies reported before. Since changes in the biochemical composition of the meat carry the potential to result in an accelerated microbial growth, a more comprehensive approach to characterize the effects from farm to fork is required. Thus, the aspects of husbandry conditions, animal health and welfare are not limited to ethical concerns. In fact, potential effects should be investigated under consideration of the quality and also shelf life of the end product.

5.7. Conclusion

This study revealed significant effects of husbandry conditions on meat quality and shelf life, even at the early stages of production. The rearing and fattening process of pigs influenced typical meat quality parameters with consequences for the spoilage process and shelf life. Antibiotic medication or health status showed no significant effect on microbial shelf life, even though the results for particular clinical findings indicated a

reduced microbial shelf life for animals suffering from illness. Further investigations are needed to elucidate the underlying effects between husbandry, animal health and shelf life.

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6. Characterization of the Meat Quality and Spoilage of Fresh Pork Meat in Relation to Terminal Sire Genotype Selection

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To be submitted

6.1. Abstract

The influence of terminal sire genotype (German Piétrain and PIC 408) on carcass characteristics, meat quality and shelf life was investigated and compared to a Control group within a commercially used pig population. All piglets were reared together in a conventional pig production system and slaughtered in an industrial slaughterhouse. Carcass characteristics were assessed by an AutoFOM system in the slaughterhouse, nutritional value of the meat was determined by near-infrared spectroscopy (NIRS). For 155 pigs, typical meat quality parameters (pH, drip loss, color, aw-value) were investigated on slices of pork loin stored aerobically at 7°C in the laboratory. Microbial and sensory investigations were conducted to determine the shelf life of the pork slices. Higher lean meat percentage as well as ham and shoulder cuts were observed for the selected terminal sires in comparison to the Control group. The content of collagen and intramuscular fat was higher for the Control group. The German Piétrain group showed a lower meat quality with higher incidence for pale soft and exudative meat. The PIC 408 group showed a better meat color and higher pH-values in comparison to both other groups. Sensory as well as microbial shelf life was 7 d and did not differ between groups. The parameter 'purchase decision' was not affected by differences in meat quality among the samples. The results indicated a higher susceptibility to stress for the German Piétrain group caused by particular genetic characteristics of the line.

6.2. Introduction

The global consumption for animal-based protein, especially for meat, increased remarkably in the last decades (Sans and Combris, 2015). Besides poultry, pig meat showed the highest increment of growth on a global scale with an enduring pressure on meat industry to produce affordable products. As the meat market reached the saturation point in Europe, meat quality became an increasingly important driver for the consumer choice while the effect of income and price of the product is declining (Verbeke et al., 2010; Henchion et al., 2014). Besides, there is a rising consumer awareness on product safety, animal health and welfare as well as a sustainable production (Verbeke and Viaene, 2000). Thus, the pork meat industry faces the challenge of meeting consumer demands and concerns but also remaining competitive on the major markets. As a result, a high variety of pork production systems and distribution chains emerged in Europe (Trienekens et al., 2009; Verbeke et al., 2010). Several efforts have been made

to enhance meat quality and performance within pork meat production chains. The variation of different growing intensities, production systems and outdoor access were reported to have implicit effects on carcass characteristics and meat quality (Olsson and Pickova, 2005; Hansen et al., 2006; Bonneau and Lebret, 2010). The pre-slaughter stress, transportation times and stocking densities affect the glycolytic potential of the muscle and thus meat quality (Rosenvold and Andersen, 2003b; Gajana et al., 2013). Additionally, pig nutrition has the ability to determine the fatty acid profile, protein acretion as well as lean meat content and marbling (Pettigrew and Esnaola, 2001). Besides, also sex and breed has to be taken into account (Olsson and Pickova, 2005; Jeleníková et al., 2008). Since combinatory effects may compensate or reinforce each other, the identification of direct consequences on meat quality within whole production systems is difficult. The intercorrelation of meat quality traits indicate that changes in particular quality characteristics affect many other meat quality attributes and thus also shelf life (Huff-Lonergan et al., 2002). Thus, a detailed view on the particular animal specific factors influencing pork meat quality and how they interact is mandatory (Rosenvold and Andersen, 2003a). For the biochemical composition, the leanness, palatability and ultimate quality of the meat, the genotype is one important factor (Brewer et al., 2002; Wood et al., 2004; Lee et al., 2010, 2012). The muscle fiber characteristics are influenced by the breed as well, with consequences for postmortem metabolic rate and deterioration of meat quality parameters (Maltin et al., 2003; Ryu and Kim, 2005). New developments in the meat market are often realized by an optimization of breeding to meet both, the consumer demands for enhanced meat quality as well as an efficient production (Ngapo and Gariepy, 2008; Trienekens et al., 2009). The variation of terminal sire genotypes effects the dressing percentage, intramuscular fat content, tenderness and color of the meat filets (Latorre et al., 2003). For terminal pig production, crossbreeding allows for the combination of heterogene and also complementary characteristics. Their impact on carcass characteristics and initial meat quality is well investigated. But the developement of typical meat quality parameters during storage is often not considered, even though a few studies indicate a relationship between animal specific parameters and shelf life (Huff-Lonergan et al., 2002; Ryu and Kim, 2005; Faucitano et al., 2010). Thus, more research is needed on terminal genotype dependent meat quality parameters and their stability during storage. The aim of the study was a comparison of two terminal sire lines and one Control group within a commercially used pig population. In a conventional production system, their influence on carcass characteristics, meat quality and shelf life should be investigated. Two premium sire lines were selected to optimize meat

quality regarding a better marbling and an increased consumer acceptance.

6.3. Material and Methods

In total, 155 pigs were investigated during the trial. Breeding and fattening of the pigs was conducted in mixed pens. Rearing and fattening was conducted in West Germany under standardized conditions. The sows were Danbred and the boars of the conventional production line were Piétrain. Premium sires of German Piétrain and PIC 408 were selected for breeding the pigs for the trial. According to the producer (GFS Topgenetik), the premium sires are characterized by enhanced meat content and higher daily weight gains with 521 g (PIC 408) and 502 g (German Piétrain). The pigs were slaughtered in 10 batches in the same industrial slaughterhouse at a final weight of 96 kg. Processing of the carcasses was conducted 24 h after slaughter at a German pig processor. After deboning and dissection, the fat covering was removed from the *Musculus longissimus dorsi* and the sample was cut in 3 cm broad slices. Filets were packed aerobically and transported in insulated boxes under temperature-controlled conditions to the laboratory of the University of Bonn. In the laboratory, the filets were separated and packed aerobically as single items in polyethylene trays with a snap-on lid. The samples were stored at 7°C in low-temperature, high precision incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan). The storage temperature was monitored by data loggers (ESCORT JUNIOR Internal Temperature Data Logger, Escort, New Zealand), with measurements taken every 5 min.

6.4. Study Design

First investigations started directly after arrival in the laboratory, 24 h postmortem. The samples were stored for 240 h (10 d) with investigation points every two days. Except for the parameters nutritional value and drip loss, the physicochemical properties, microbial load and sensory investigations were conducted at every investigation point during storage to characterize meat quality and assess shelf life. For the determination of the nutritional value, one filet per animal was frozen at -18°C at the first investigation point (24 h postmortem).

6.4.1. Physicochemical Parameters

The physicochemical parameters comprised the carcass characteristics, nutritional value, pH-value, drip loss, water activity and color measurements. Based on the measurement results, meat was classified as pale, soft and exudative (PSE) meat when the following criteria were observed: pH$<$5.5, L*-value$\geq$ 51 and drip loss $\geq$ 2. The criteria were modified according to the definition by Faucitano et al. (2010) and Gajana et al. (2013). Carcass characteristics were measured in the slaughterhouse by the full automatic ultrasound system Autofom (Busk et al., 1999). Based on the values for fat measure and meat measure, the lean meat percentage (LMP) of the carcass was calculated using the following equation (6.1, European Union (2011)):

$$LMP = 58.10122 - 0.56495 \cdot F + 0.13199 \cdot M \tag{6.1}$$

with LMP: estimated percentage of lean meat in the carcass, F — fat measure, M — meat measure.

The measurements comprised intramuscular fat, protein, water and collagen content and are stated as percent. The analyses were conducted using near-infrared spectroscopy (NIRS). After defrosting at 4°C for 24 h, meat samples were chopped using a food processor (Tefal La Mulinette, Groupe SEB Deutschland GmbH, Offenbach am Main, Germany). The meat paste was placed in a spectrometer (NIRS DS2500, Foss, Rellingen, Germany) and analyzed automatically.

Measurement of pH-value

The pH-value was measured on the meat surface at every investigation point, using a portable pH-meter (pH 8011, Peter Bock Umwelttechnik, Gersfeld, Germany). Three measurements were performed for each meat sample by placing the electrode onto the meat surface. An average pH-value was calculated for every meat slice.

Color Measurements

Color measurements were conducted using a high-precision, lightweight chromameter with a measuring head of 8 mm (KonicaMinolta CR 400). The device works with a wavelength between 400 nm and 700 nm and a 45°/0° geometry. The device matches a CIE 1931 standard observer, measured with D65 illuminant (6500 K daylight). The L*a*b*-values as well as C* and h°-values were recorded for each filet at three sample

points on the meat surface to get a representative evaluation of the samples. Measurements were averaged for each filet. As a measure for a fresh pink meat color, the Redness Index (RI) was calculated with the following equation (6.2):

$$RI = \frac{a*}{b*} \tag{6.2}$$

Water Activity

The water activity (a_w-value) of the meat samples was measured using a LabMaster-a_w device (Novasina, Lachen, CH). Thin slices of the muscle tissue were cut and transferred to a small plastic tray using a scalpel and tweezers until the bottom of the measurement tray was completely covered. After pre-heating of the sample in the measurement device, measurement of the a_w-value was conducted at 25°C, until the measured value was stable for at least 1 min.

Measurement of Drip Loss

The measurement of the drip loss was conducted 24 h postmortem by using the bag method. The filets were hung on hooks and placed in plastic bags. The bags were sealed with rubber bands to avoid evaporation and then hung from a grate for 24 h in a 4°C incubator. The samples were weighed before and after hanging. Drip loss was calculated as the difference in weight, corrected for mass and expressed as percent (equation 6.3).

$$D_L = \frac{m_1 - m_2}{m_1} \cdot 100\% \tag{6.3}$$

Where DL is drip loss [%], m_1 is mass before hanging and m_2 is mass after hanging.

6.4.2. Microbial Investigations

For assessing total viable count (TVC), 25 g of the meat surface tissue with a size of 4 x 7 x 0.5 cm, was aseptically taken using a sterile scalpel. The sample was transferred to a filtered stomacher-bag (Interscience, Saint Nom la Bretèche, FR) and filled with 225 ml saline peptone diluent (0.85% NaCl with 0.1% peptone; Oxoid, Basingstoke, United Kingdom). The sample was homogenized for 60 s using a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany). The homogenate was used to prepare a 10-fold dilution series using saline peptone diluents. Appropriate dilutions were transferred to plate count agar (PCA, Oxoid, Basingstoke, United Kingdom) and incubated at 30°C

for 72 h.

6.4.3. Sensory Investigations

Sensory investigations were conducted by a trained sensory panel with four panelists. The analyses comprised the purchase decision (PD) and sensory investigations of the freshness. The PD was requested via a dichotome response option prior to opening the packaging to avoid a bias by the odor. The freshness of the samples was evaluated based on a graded five-point-scoring system with five meaning highest quality and one meaning spoiled. The evaluation was performed for the parameters color, odor, texture and meat juice emersion for each sample. The Sensory Index (SI) was calculated as a weighted average with the following equation (6.4)

$$SI = \frac{2 \cdot O + 2 \cdot C + T + J}{6} \tag{6.4}$$

Where SI is Sensory Index, O is odor, C is color, T is texture and J is meat juice.

According to the scheme, the product is spoiled when the SI reaches the level of 2.3. The SI was plotted as a function of time and fitted to a linear model. Thus, the shelf life of each sample was calculated by equation 6.5 as follows (Kreyenschmidt, 2003):

$$SL = \frac{2.3 - a}{b} \tag{6.5}$$

Where SL is shelf life, a is the intercept of the linear model and b is the slope of the linear model.

6.4.4. Statistical Analysis

Data were tested for normal distribution using a K-S test. Since the data did not meet the conditions for parametric testing, non-parametric statistical tests were used. The differences between groups were tested with a Kruskal-Wallis test with Bonferroni correction for pairwise comparisons. Correlation analyses were conducted with a Spearman's Rank correlation.

Microbial growth was fitted as a function of time using nonlinear regression (Levenberg-Marquardt algorithm). The modified Gompertz model was used to describe the logistic growth curve (Gibson et al., 1987):

$$N(t) = A + C \cdot e^{-e^{-B \cdot (t-M)}} \tag{6.6}$$

With N(t): microbial count log_{10} [cfu/g] at time t, A: lower asymptotic line of the growth curve (N0 = initial bacterial count log_{10} [cfu/g]), C: difference between upper asymptotic line of the growth curve (Nmax = maximum population level log_{10} [cfu/g] and the lower asymptotic line (A log_{10} [cfu/g]), B: relative growth rate at time M [1/h], M: time at which maximum growth rate is obtained (reversal point), t: time [h].

The end of shelf life was achieved when total viable counts reached 7.5 log_{10} cfu/g. All data analyses were conducted using SPSS 24 (IBM SPSS Statistics, USA), Origin Pro 8G (OriginLab Corporation, Northampton, MA) and R (r-project.org).

6.5. Results

Carcass characteristics and mean values of meat cuts are displayed in Table 6.1. The carcass weight, belly and shoulder cuts showed no significant differences between all investigation groups. The ham and loin cuts as well as LMP were significantly smaller

Table 6.1.: Carcass characteristics and meat cuts of the investigated groups

	Control (n=90)	German Piétrain (n=38)	PIC 408 (n=26)
Carcass weight [kg]	95.68±4.15	96.41±3.33	97.20±2.98
Ham [kg]	19.02±1.04^a	19.52±0.81^b	19.60±0.84^b
Loin [kg]	7.56±0.48^a	7.79±0.41^b	7.87±0.38^b
Belly [kg]	13.17±1.04	12.89±1.01	13.33±0.71
Shoulder [kg]	9.25±0.44	9.40±0.32	9.39±0.39
LMP [%]	59.88±1.04^a	60.37±1.22^b	60.28±0.96^{a,b}
Sex	60f/30m	32f/6m	23f/3m
Clinical findings (n)	3	11	1
PSE cases (n)	1(1.1%)	5(13.2%)	0

for the Control in comparison to both other groups. No significant differences in carcass characteristics or meat cuts were observed between German Piétrain and PIC 408 but both groups showed significantly higher values for ham and loin in comparison to the Control group. For German Piétrain, clinical findings were significantly higher than for both other groups. The results of the nutritional analyses for the particular investigation groups are stated in Table 6.2.

Table 6.2.: Nutritional value of fresh pork loin of the investigated groups

	Control (n=90)	Germain Piétrain (n=38)	PIC 408 (n=26)
Protein [%]	24.48±0.47	24.54±0.44	24.40±0.46
Collagen [%]	0.75 ± 0.15^{a}	0.68 ± 0.13^{b}	0.69 ± 0.13^{ab}
Intramuscular Fat [%]	1.79 ± 0.58^{a}	1.54 ± 0.33^{b}	1.58 ± 0.38^{ab}
Water [%]	73.63±0.56	73.72±0.44	73.83±0.61

No difference between groups was found for the protein and water content. The collagen as well as intramuscular fat content of the Control group was significant higher than of German Piétrain, but no difference was observed between German Piétrain and PIC 408. The investigated meat quality parameters and their development during storage are displayed in Table 6.3.

Table 6.3.: Quality parameters of pork loin and their deterioration during storage

Storage time	0 h	48 h	96 h	144 h	168 h	240 h
pH						
Control	5.58 ± 0.16^{a}	5.49 ± 0.14^{a}	5.51 ± 0.15^{a}	5.56 ± 0.19^{a}	5.63 ± 0.28^{a}	6.25 ± 0.62^{a}
German Piétrain	5.53 ± 0.09^{a}	5.42 ± 0.08^{b}	5.42 ± 0.10^{b}	5.45 ± 0.10^{b}	5.49 ± 0.16^{b}	5.78 ± 0.43^{a}
PIC 408	5.63 ± 0.07^{b}	5.52 ± 0.10^{a}	5.52 ± 0.10^{a}	5.54 ± 0.11^{a}	5.53 ± 0.12^{ab}	6.09 ± 0.50^{b}
L*						
Control	49.01 ± 2.51^{a}	50.30 ± 1.92^{a}	49.91 ± 2.71^{a}	49.44 ± 2.56^{a}	49.92±2.53	50.20±2.46
German Piétrain	50.73 ± 1.66^{b}	51.26 ± 2.12^{b}	51.53 ± 2.73^{b}	49.54 ± 3.80^{a}	49.57±1.52	50.57±1.66
PIC 408	48.49 ± 2.10^{a}	49.81 ± 1.16^{a}	50.46 ± 1.13^{ab}	51.05 ± 1.58^{b}	50.53±1.89	49.30±2.65
a*						
Control	5.09±1.10	5.25 ± 1.07^{a}	4.74 ± 1.18^{ab}	4.96±1.16	4.81 ± 0.99^{a}	4.49 ± 1.05^{a}
German Piétrain	5.27±1.06	4.69 ± 0.88^{b}	4.31 ± 0.96^{a}	5.22±1.08	5.52 ± 0.71^{b}	5.28 ± 0.74^{b}
PIC 408	5.48±0.95	5.33 ± 0.89^{a}	5.09 ± 0.70^{b}	4.66±0.63	4.36 ± 0.65^{a}	4.52 ± 1.16^{a}
b*						
Control	6.57 ± 1.53^{a}	7.07 ± 0.78^{ab}	7.39 ± 1.15^{a}	6.79±1.68	6.62 ± 0.97^{a}	7.39 ± 1.07^{a}
German Piétrain	7.13 ± 0.74^{b}	7.31 ± 0.79^{a}	7.67 ± 0.87^{a}	7.19±1.91	6.14 ± 0.77^{a}	6.89 ± 0.71^{a}
PIC 408	5.09 ± 1.01^{c}	6.69 ± 0.71^{b}	6.85 ± 0.61^{b}	6.96±0.57	7.37 ± 0.71^{b}	7.81 ± 1.47^{b}
aw						
Control	0.983±0.006	0.982 ± 0.006^{a}	0.984±0.005	0.983±0.004	0.980±0.004	0.984±0.005
German Piétrain	0.984±0.006	0.997 ± 0.003^{b}	-	-	0.980±0.003	0.983±0.005
PIC 408	0.980±0.002	0.981 ± 0.004^{a}	0.980±0.006	0.985±0.007	0.985±0.009	0.984±0.007
Drip loss [%]						
Control	1.70 ± 0.96^{a}					
Germain Piétrain	2.33 ± 1.12^{b}					
PIC 408	2.03 ± 1.06^{ab}					

The initial pH-values were between 5.53 (German Piétrain) and 5.63 (PIC 408). The PIC 408 group showed significantly higher initial pH-values in comparison to both other groups. The pH-values remained stable during storage, but showed a significant increase at the last investigation point. The mean pH-values were between 5.78 (German Piétrain) and 6.25 (Control) when the meat was spoiled. From the beginning until the end of storage, filets of German Piétrain showed the lowest pH. This effect was significant for every investigation point except for the first one. Highest pH-values were observed

for the PIC 408 group until 144 h of storage, but the Control group showed highest pH-values for the last two investigation points. A PSE like condition occurred for German Piétrain filets (13.2%) and the Control group (1.1%), but not for PIC 408. The initial mean L*-value was between 48.49 (PIC 408) and 50.73 (German Piétrain). The L*-value was significantly higher for German Piétrain in comparison to both other groups (Table 6.3). Filets of German Piétrain showed highest mean L*-values until 96 h of storage. L*-values were fluctuating during storage and showed no clear development. When the meat was spoiled, L*-values between 49.30 (PIC 408) and 50.20 (Control) were recorded. Significant differences between groups showed no consistent tendency.

The mean initial a*-values for the fresh filets were between 5.09 (Control) and 5.48 (PIC 408), with no significant differences between the groups. As for the L*-values, a*-values were fluctuating during storage and showed no clear trend. Significant differences between groups are stated in Table 6.3. The measured differences were scattered and without a clear pattern of a particular group showing higher or lower a*-values than the others.

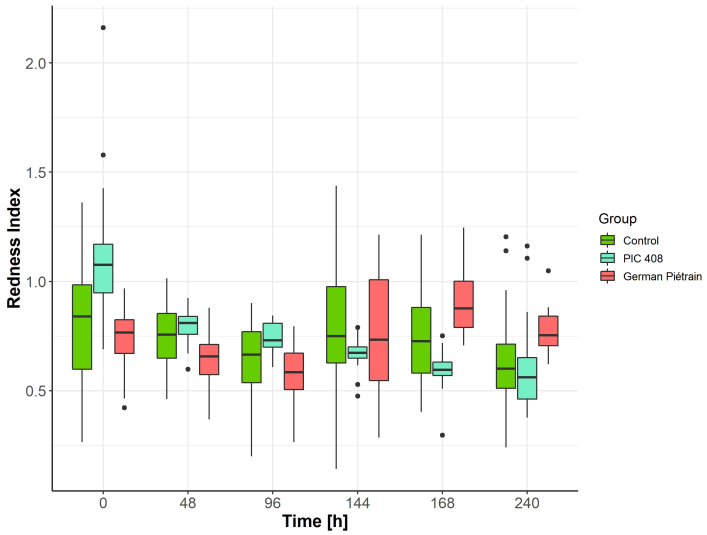

Figure 6.1.: Development of the Redness Index during storage for all investigation groups

The b*-values were between 5.09 and 7.13 at the beginning of storage. The b*-values were highest for German Piétrain until 168 h of storage and then dropped remarkably to display the lowest values of all investigated groups. For PIC 408, it was the opposite with lowest b*-values in the beginning changing to highest values at the end of storage. The development of b*-values during storage showed a significant increase for PIC 408. For

the Control group, b*-values were fluctuating during storage and showed no clear trend. The Redness Index was significantly higher for the PIC 408 group at the beginning of storage and until 96 h (Figure 6.1). The RI of the PIC 408 filets showed a steady decrease during storage, indicating a loss of the color typical for fresh meat. RI-values of the other groups decreased until 96 h, afterwards the RI showed a broad scattering and no clear tendency for all groups.

The initial mean a_w-values for the investigated groups were between 0.980 (PIC 408) and 0.984 (German Piétrain). The a_w-values showed no significant difference between groups or development over the storage time. All measured a_w-values were in a narrow range with no measurement below 0.980. The drip loss values were between 1.70% (Control) and 2.33% (PIC 408). The drip loss of Germain Piétrain was significantly higher in comparison to both other groups. The pork loins showed an initial mean TVC

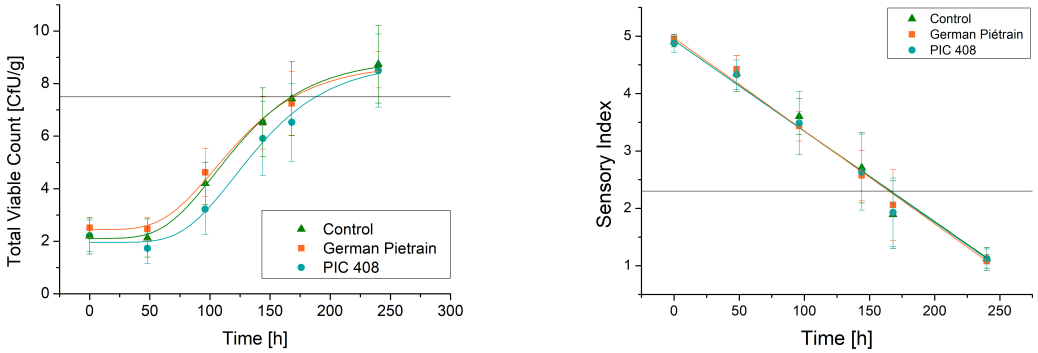

Figure 6.2.: Assessment of microbial and sensory shelf life of all investigation groups

contamination between 2.20 $\log_{10}$ cfu/g and 2.52 $\log_{10}$ cfu/g (Figure 6.2, Table 6.4). The initial contamination was significantly higher for German Piétrain in comparison to both other groups. For German Piétrain, the lag time showed significantly lower values in comparison to the PIC 408 group while the Control group was in between. The maximum growth rate of microorganisms showed no significant difference between all investigated groups. Additionally, no difference was detected for the maximum values of TVC. The significantly longer lag time for the PIC 408 group led to a later intersection with the acceptance level, but this difference is not significant. The mean microbial shelf lives were 170 h (Control), 179 h (German Piétrain) and 181 h (PIC 408).

The filets showed a high sensory quality upon arrival at the laboratory (Figure 6.2). The initial SI was between 4.88±0.16 (PIC 408), 4.91±0.09 (Control) and 4.95±0.07

Table 6.4.: Microbial growth parameters of all investigation groups

	Control (n=90)	German Piétrain (n=38)	PIC 408 (n=26)
Initial TVC $\log_{10}$ cfu/g	2.20 ± 0.69^{a}	2.52 ± 0.38^{b}	2.21 ± 0.61^{a}
Maximum TVC $\log_{10}$ cfu/g	8.84 ± 1.187	8.57 ± 0.695	8.50 ± 1.394
t_{lag}	63.77 ± 22.89^{ab}	55.12 ± 23.08^{a}	73.71 ± 28.52^{b}
xc	111.61 ± 22.35^{a}	108.95 ± 21.58^{a}	127.46 ± 27.21^{b}
μmax	0.0695 ± 0.034	0.0599 ± 0.037	0.0767 ± 0.053
Microbial Shelf life [h]	170.41 ± 40.93	178.94 ± 39.84	180.62 ± 40.07
Sensory Shelf life [h]	175.89 ± 23.36	175.28 ± 15.65	170.73 ± 28.08

(German Piétrain). There was no significant difference of the SI between the investigated groups at any point of investigation. The progress of the deterioration was very similar for all groups. The estimated sensory shelf lives were 171 h (PIC 408), 175 h (German Piétrain) and 176 h (Control) with no significant difference between groups. The purchase decision (PD) showed high values upon arrival at the laboratory with over 95% positive PDs for the investigated filets (Figure 6.3).

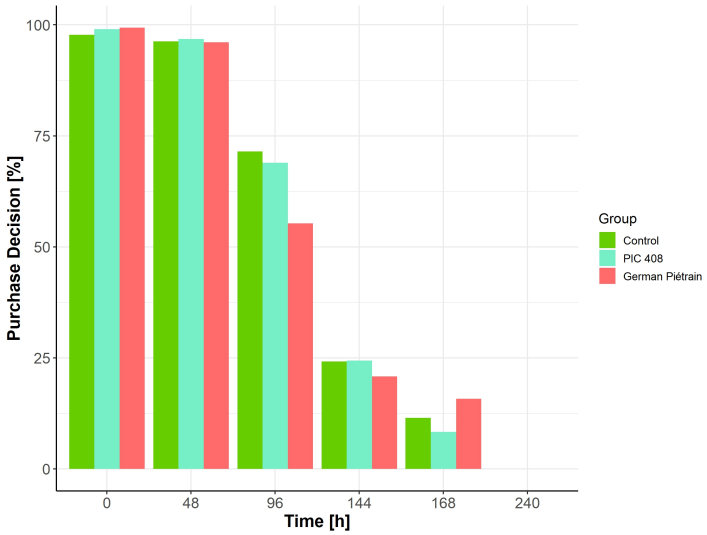

Figure 6.3.: Development of the purchase decision during storage for all investigation groups

The mean initial PD were 97.8%±10.13 (Control), 99.0%±4.90 (PIC 408) and 99.3%±4.01 (German Piétrain). There was no significant difference between the investigation groups until 48 h of storage. At 168 h, more than 80% of the filets were rejected due to deterioration and no positive PD was recorded at the end of storage. The panel showed no significant preference for one of the investigated groups over the whole storage time. Regression analysis revealed, that the PD was mainly determined by the meat color in

the sensory evaluation (k:0.918, p<0.001).

The correlation analysis revealed medium and low correlations for the physicochemical parameters of the fresh pork filets (Table 6.5). Beyond that, intercorrelations between the color values or microbial modelling parameters were observed. Regarding the meat composition, high water contents were related to a lower protein content and low fat content. Additionally, the protein content was negatively correlated to the collagen content as well as intramuscular fat. The pH-value was negatively correlated to the a_w-value, drip loss and protein content of the filets. Thus, the German Piétrain group showed the lowest pH-values and also highest drip loss as well as a_w. Sensory shelf life and microbial shelf life showed a high positive correlation (k: 0.727, p<0.001), indicating that microbial and sensory freshness indicators correspond well.

Table 6.5.: Spearman's ρ correlation of physicochemical parameters at the beginning of storage. Bold values are significant at the 0.05-level.

	Protein	Water	Fat	Collagen	pH	Aw	Drip loss
Water	***-.430***						
	.000						
Fat	**-.203**	***-.449***					
	.015	***.000***					
Collagen	**-.202**	-.134	.128				
	.016	.112	.129				
pH	***-.300***	***.335***	.028	.123			
	.000	***.000***	.743	.144			
A_w	**.266**	**-.240**	.137	-.079	***-.364***		
	.018	**.033**	.230	.487	***.001***		
Drip loss	.133	-.009	**-.180**	-.090	***-.268***	.097	
	.115	.913	**.032**	.286	***.001***	.393	

6.6. Discussion

The application of German Piétrain and PIC 408 as terminal sire genotypes led to a higher content of LMP as well as larger ham and loin cuts in comparison to the Control group. Other carcass characteristics or carcass weight were not affected. The nutritional composition of the pork filets analyzed in this trial was in accordance with former studies (Kim et al., 2008). The filet composition was very similar for all investigated groups. Even though the breed is able to determine muscle fiber characteristics, marbling and intramuscular fat content (Wood et al., 2004; Ryu et al., 2008; Lee et al., 2012), this goal was not achieved via the optimized sire selection during this trial. In contrast, the Control group showed higher intramuscular fat content and a lower LMP during this trial. An improvement for these meat quality parameters was not achieved within the

Piétrain breed according to the results of this study. Crossbreeding with Duroc led to promising improvements of marbling in former studies (Channon et al., 2004; Mörlein et al., 2007). Thus, other breeds besides Piétrain should be considered for enhancing marbling and intramuscular fat content in commercial pig production. In general, the investigated samples showed a very high quality upon arrival at the laboratory. The pH-value was in the normal range for pork filets (Faucitano et al., 2010; Bruckner et al., 2012) and showed a typical increase at the end of storage due to the accumulation of microbial metabolites on the meat surface (Gill, 1983; Bruckner et al., 2012). Even though the German Piétrain group showed significantly lower pH-values than both other groups, the differences are low and implicate no effects on technological traits or shelf life. According to the measurements for the L*-value, meat color of all investigation groups was in a normal range (Warriss and Brown, 1995; Faucitano et al., 2010) with no indication for dark firm dry (DFD) meat and only a few cases of PSE meat (Faucitano et al., 2010; Gajana et al., 2013). Significant meat color differences between pure breeds were reported before (Lindahl et al., 2001; Brewer et al., 2002), but a specific effect of the terminal boar genotype is controversial (Latorre et al., 2003). The high L*-values and low RI point to a more pale and less red meat color of German Piétrain group in comparison to the other groups. Since also the drip loss and pH-value were different, the results indicate higher muscle glycogen stores of the pigs. The glycolytic potential of the muscle has been reported to be influenced by diet, husbandry and pre-slaughter handling (Pettigrew and Esnaola, 2001; Rosenvold and Andersen, 2003a). Given that these factors were the same for all animals during this trial, a higher susceptibility to pre-slaughter stress is supposed for the German Piétrain group due to genetic characteristics. In fact, heterozygos and homozygos carriers of the halothane gene are typical for Piétrain populations (Garnier et al., 2003; Stratz et al., 2014). The halothane gene is related to the porcine stress syndrome (PSS), an enhanced lean meat content and a higher incidence of PSE meat due to a rapid pH drop postmortem (Fàbrega et al., 2002; Rosenvold and Andersen, 2003a; Salas and Mingala, 2017). This is in accordance with the findings of this study where the German Piétrain also showed a significantly higher incidence of PSE meat, highest LMP and more clinical findings at the abattoir. Thus, optimization of the terminal sire line within the Piétrain genotype still carries the risk of producing less stress resistant animals with implicit consequences for meat quality. Significantly higher values of initial microbial contamination in the German Piétrain group could be related to the higher counts of clinical findings at the abattoir. However, these results did not affect microbial or sensory shelf life of the filets. The shelf life of all investigated

groups showed no significant differences and was longer in comparison to former studies due to low initial microbial counts (Blixt and Borch, 2002; Liu et al., 2006; Bruckner et al., 2012). The slower microbial growth on filets of the PIC 408 can be explained by lower microbial counts at the beginning of storage. In general, the results of the PD assessment showed a high acceptance of the filets and no significant preference for a particular investigation group. Thus, differences in meat color of intramuscular fat content did not affect the PD as it was shown in former studies (Ngapo et al., 2004; Font-I-Furnols and Guerrero, 2014).

6.7. Conclusion

The investigation showed that improving meat quality and consumer acceptance was not achieved by optimizing terminal sire genotypes within the Piétrain breed. For an enhanced marbling or intramuscular fat content, cross breeding with other genotypes, e.g. Duroc, was proposed. For optimization within the Piétrain breed, the incidence of animals susceptible to stress and occurrence of PSE meat still has to be taken into account. Nevertheless, microbial as well as sensory shelf life was not affected by optimizing terminal sire genotypes.

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7. General conclusion

A high quality and storage stability of fresh meat is essential for the supply of goods in increasingly complex production chains. With a growing animal production and an oversupply of fresh meat in the last decades, the consumer demands relocated from low price to high quality products. Additionally, ethical concerns about animal welfare and health, safety as well as the sustainability of production arose among consumers. Therefore, the meat industry strives to satisfy refining consumer demands while remaining competitive on the major markets. During the optimization of animal production, the influence of animal specific factors on meat quality is often investigated directly after slaughter. But their influence on the storage stability, or rather shelf life, is often not considered, as well as comprehensive approaches from farm to fork. Thus, this study aimed at the investigation of animal specific factors and their influence on the quality and shelf life of fresh poultry and pork meat. Several trials in experimental as well as industrial set ups were conducted to elucidate the research questions.

First, the influence of methionine supplementation in broiler diet on certain meat quality and shelf life parameters was investigated. Since the dietary composition has the potential to determine the oxidative status and thus biochemical composition of the meat, effects of the supplementation of three different concentrations and two sources (DLM and DL-HMTBA) of this essential amino acid were investigated. The study revealed that the dose of methionine supplementation, but not the source, had a significant impact on meat quality, especially in comparison to the basal group. Methionine supplementation led to higher pH-values and drip loss in this experimental set up. The sensory investigations revealed a lower sensory quality for the basal group, but no relevant differences in shelf life. The reduced sensory quality of the basal group was related to a deteriorated oxidative status of the animals and atypical spoilage process with more emphasis on biochemical degradation. In general, microbial contamination of the filets was very low and the microbial rejection limit was not achieved by most of the samples after 192 h of storage. At the end of storage, the concentration of methionine supplementation was positively correlated to microbial counts of TVC and *Pseudomonas spp.*

pointing to an accelerated microbial growth. This effect was only observed during the first study, in an experimental set up, and was possibly caused by the effect of methionine supplementation on the pH-value and drip loss. However, husbandry of the animals, processing and cooling conditions postmortem as well as initial microbial contamination was not representative for industrial poultry production. In an industrial set up, the first trial was repeated using the same methionine sources (DLM and DL-HMTBA) for the supplementation, but with lowered concentrations. Next to the parameters investigated in the first trial, an assessment of the meat color and cooking loss was conducted. Additionally, the meat failure White Striping and the purchase decision was investigated. Due to the adjusted methionine doses, the industrial slaughter and processing, not all findings were in accordance with the former trial. In general, an effect of different methionine concentrations, but no effect of the methionine source, on meat quality were identified. The increasing effect on pH-value was confirmed, but drip loss and cooking loss of the fresh filets were reduced as a function of the methionine concentration. The methionine concentration significantly influenced meat color in the terms of darker filets with a lesser red and higher yellow proportion. The sensory shelf life was significantly shorter for the DLM group, but the difference was below 24 h and thus judged not relevant for the poultry industry. In general, filets of the methionine supplemented groups showed significantly decreased values for the purchase decision. This was mainly caused by the aberrations in color and the occurrence of White Striping on the filets. White Striping lowered the sensory acceptability of the filets. Due to an unappealing color, some filets were rejected, even though the spoilage level was not achieved yet. The occurrence of White Striping could be related to a heavy breast filet weight and methionine supplementation.

Consequently, the optimization of dietary composition, as the first research question, was proved to significantly influence meat quality and in parts also microbial growth and shelf life of the filets. An accelerated growth rate of the chicken carried the risk to provoke a higher occurrence of White Striping with negative implications for consumer acceptance and purchase decision. White Striping is a meat failure causing high financial losses due to reduced consumer acceptance and was related to heavy filet weights and fast growth rates of animals in several studies. Thus, the poultry industry aims at changing the production to breeds less susceptible for White Striping or adjusted feeding and more extensive production systems.

For answering the second research question, two different rearing lines within industrial poultry production were investigated. An alternative production line with a

decelerated growth, maize-based diet, enhanced animal welfare and no antibiotic medication was compared to a conventional production line. Biochemical composition of the breast filets, typical meat quality parameters as well as microbial counts during storage were analyzed. Furthermore, the microbial and sensory shelf life, occurrence of White Striping and purchase decision were investigated. The study revealed, that there were significant differences between both production lines for the biochemical composition of the meat. The alternative production showed benefits for the nutritional profile of the filets. Additionally, the content of L-lactic acid and D-glucose was increased in comparison to the conventional production. Differences in the biochemical composition did not lead to an accelerated growth of typical spoilage bacteria. Additionally, the filets from the antibiotics-free alternative production showed no difference in initial contamination or microbial growth in comparison to the conventional production line. Thus, no difference in microbial or sensory shelf life was observed between the investigation groups. The color of the filets from the alternative production line displayed a higher amount of yellow, but these differences did not affect purchase decision. The highest severity of White Striping was observed most for the conventional production line, but the difference was not significant. In conclusion, the alternative production system carries the potential for a more sustainable poultry production focusing on a decelerated growth, enhanced welfare of the animals and abandonment of antibiotics. Thus, an adjustment to more extensive production systems with less environmental impact is possible without any negative implications for meat quality as well as shelf life.

A more detailed investigation of the animal welfare and performance parameters was conducted for pork production in an experimental set up. Rearing of the animals, early performance as well as fattening parameters were recorded for pork of two different crossbreeds. Typical meat quality traits as well as the health status of the animals was assessed after slaughter at an industrial slaughterhouse. The microbial and sensory shelf life was investigated as a function of animal specific factors, performance parameters and meat quality traits. The study revealed the influence of particular animal specific factors on meat composition with direct effects on microbial as well as sensory shelf life. The shelf life was not affected by the breed and sex of the animals. But specific performance parameters, such as the weaning age and time spent in the fattening unit showed major impacts on the quality and shelf life of the end product. Thus, a later weaning of the animals and more time to grow in the fattening unit resulted in a prolonged microbial shelf life. These animal specific factors influenced the shelf life mainly via the determination of the content of intramuscular fat and water in the meat, leading

to an accelerated microbial growth due to higher water contents in the filets. Antibiotic medication or the health status of the animals showed no significant impact on meat quality or the shelf life of the filets. But, a tendency to a lowered shelf life was identified for animals with clinical findings at the abattoir.

The influence of terminal sire line selection on typical pork meat quality and shelf life was investigated in an industrial set up. Two sire lines and one Control group of a commercially use Piétrain population were analyzed regarding their influence on carcass characteristics, meat quality and microbial as well as sensory shelf life. The breed showed a significant impact on the lean meat content of the animals as well as pH-value and drip loss of the pork loin. During the trial, the German Piétrain group displayed a lower meat quality with higher incidence for pale, soft an exudative meat. The PIC 408 group developed a better meat color and higher pH-value in comparison to the other groups. The heterogeneity of carcass characteristics and meat quality parameters did not result in different estimations for sensory as well as microbial shelf life among the groups. The purchase decision was not affected as well. However, the results indicated that optimization within the Piétrain breed carries the risk of producing animals with a higher susceptibility to stress caused by specific genetic characteristics of the line. For an increased intramuscular fat content and improved consumer acceptance, crossbreeding with other breeds is suggested. According to the results of this study, terminal sire line optimization within the Piétrain line had implications for meat quality with special emphasis on the incidence of stress indicated meat failures, such as PSE meat.

As a concluding remark, the interrelationship between animal specific factors and meat quality as well as shelf life has to be pointed out. According to the results of this thesis, adjusting animal production affects the biochemical characteristics of the muscle to an extent that has the potential to influence the storage stability and shelf life of the product as well. Production systems focusing on a slower growth, adjusted diet and a higher motor activity of the animals showed benefits for the nutritional profile of the meat and offer the opportunity to reduce meat failures such as White Striping. For pork, a positive influence of late weaning and slower fattening on the shelf life was shown. Thus, adjusting husbandry, considering animal health and welfare, is not limited to ethical benefits, but can also improve technological traits, consumer acceptance and microbial shelf life of the product. These effects of animal specific factors on quality, shelf life and consumer acceptance comprise the whole production chain from farm to fork and should be considered during the development of enhanced, sustainable meat production systems.

A. Appendix

A.1. Appendix for Chapter 2

Linear modeling (forward stepwise) for assessing the influence of the predictors on the Purchase Decision (AICC: -1,508.295, R^2= 0.724).

Table A.1.: Model summary effects – target variable: purchase decision

Source	Sum of Squares	df	Mean Square	F	Significance	Importance
Corrected Model	29.187	7	4.170	156.957	.000	
Color (sensory)	12.340	1	12.340	464.509	.000	0.720
White Striping	2.410	1	2.410	90.722	.000	0.141
Mean Demerits	1.477	1	1.477	55.617	.000	0.086
L*value	0.587	1	0.587	22.096	.000	0.034
Methionine supplementation	0.212	2	0.106	3.995	.019	0.012
Initial Weight	0.119	1	0.119	4.479	.035	0.007
Residual	10.892	410	0.027			
Corrected Total	40.079	417				

Table A.2.: Model summary coefficients – target variable: purchase decision

Source	Coefficient	Std. Error	Significance	Importance
Intercept	-2.331	0.294	.000	
Color (sensory)	0.734	0.034	.000	0.720
White Striping	-0.160	0.017	.000	0.141
Mean Demerits	-0.416	0.056	.000	0.086
L*value	0.021	0.004	.000	0.034
Methionine = Basal	0.018	0.030	.547	0.012
Methionine = DLHMTBA	0.049	0.017	0.006	
Methionine = DLM	0[a]			
Initial Weight	0.000	0.000	.035	0.007

[a] This coefficient was set to zero because it is redundant

Table A.3.: Meat quality parameters at the end of shelf life

	Basal	DLM 0.04	DLM 0.12	DLM 0.32	DL-HMTBA 0.04	DL-HMTBA 0.12	DL-HMTBA 0.32
pH_{192}	6.11±0.19	6.36±0.29	6.25±0.24	6.24±0.20	6.12±0.19	6.27±0.21	6.29±0.24
DL_{192}	0.251±0.22	0.299±0.18	0.234±0.14	0.232±0.15	0.244±0.17	0.232±0.19	0.257±0.25
L^*_{216}	54.10±3.06	56.03±2.98	55.16±3.19	53.5±3.08	55.43±2.78	55.99±2.66	54.35±3.37
a^*_{216}	6.82±0.89	6.75±1.22	6.83±0.9	6.57±1.06	6.63±0.87	6.53±0.98	6.38±1.09
b^*_{216}	15.82±2.2	17.14±2.13	16.47±2.01	15.63±1.79	16.67±2.06	16.86±2.55	15.8±1.76
SI_{192}	1.44±0.20	1.29±0.19	1.47±0.14	1.50±0.18	1.50±0.12	1.49±0.19	1.46±0.20

A.2. Appendix for Chapter 3

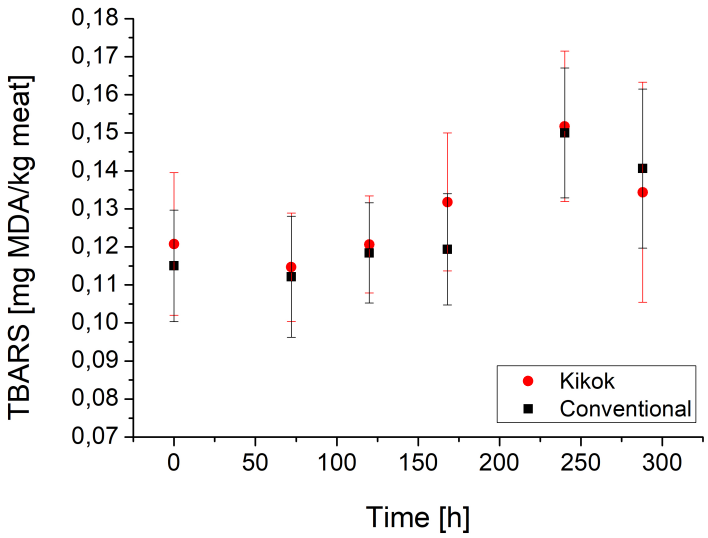

Figure A.1.: Development of TBARS during storage on Kikok and conventional produced filets

A.3. Appendix for Chapter 4

Generalized linear model to predict microbial shelf life (AICC: 731.183)

Table A.4.: Model effects for the dependent variable 'Microbial shelf life'

Model effects	Wald-Chi-Quadrat	df	Sig.
Constant	**11.048**	**1**	**.001**
Sex	**8.765**	**1**	**.003**
Breed	**7.667**	**1**	**.006**
Antibiotics	.131	1	.718
Clinical findings	2.055	1	.152
Birth weight	2.955	1	.086
Weaning age	**10.987**	**1**	**.001**
Weaning weight	.156	1	.693
Start day	.a		
Start weight	.517	1	.472
Slaughter age	.a		
Slau weight	.010	1	.920
Days in FU	.a		
ADG	.398	1	.528
FCR	2.713	1	.100
Opto	2.282	1	.131
pH_{24}	.064	1	.800
pH decline	**6.134**	**1**	**.013**
EC_{24}	**5.781**	**1**	**.016**
EC decline	.403	1	.525
IMF	.089	1	.765
H_2O	**9.949**	**1**	**.002**
Protein	3.513	1	.061
Collagen	.812	1	.368
Drip loss	.022	1	.882
Thaw loss	.304	1	.581
Cook loss	2.532	1	.112
Shear force	.000	1	.991
Initial contamination	1.295	1	.255

List of Figures

List of Tables

List of publications

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