The meat science perspective of spirulina (*Arthrospira platensis*) and black soldier fly larvae (*Hermetia illucens*) as alternative protein feeds in broiler and swine production

Dissertation

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Unofficial Title

Green Feed and Ham

Dedication

This dissertation is dedicated to my husband and my cat – Max and Joey. As is often the case, my partners in crime know me better than I know myself, and it was their confidence in me that gave me the patience and persistence to keep going.

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Synopsis

Soybean meal has been extensively researched and is currently the standard protein feed used in Western European pork and poultry production. However, in recent years concerns have arisen regarding the production of soybeans. First, soybean production has been named as a driving force of rainforest deforestation in South America. Second, increases in productivity are mostly due to the development of genetically-modified (GM) varieties, which are an anathema in many Western European markets. Third, Western Europe is highly dependent on soybean imports for securing feed for its meat and dairy supply, making it very susceptible to world market supply and price fluctuations. Additionally, the demand for animal-based proteins, and therefore protein feeds, is expected to increase globally due to a growing population and changing diets, especially in low-to-middle income countries. Increasing the productivity of current animal production systems will not be enough to ensure the availability of protein feed; therefore, alternative sources of protein feed will be needed to ensure animal production matches demand.

This dissertation, as a part of the larger Sustainability Transitions in the Food Sector project funded by Lower Saxony's Ministry for Science and Culture, investigated the impact of two alternative protein sources, spirulina (*Arthrospira platensis;* SP) and partially-defatted black soldier fly larval meal (*Hermetia illucens;* HI), on broiler chicken and pork meat quality when replacing 50 to 100% of soybean meal in animal diets. Constructive aspects of meat quality, such as physicochemical characteristics, sensory analysis, and consumer preferences, were evaluated. The results have been documented in four research articles and are summarized here.

In general, SP and HI did not compromise physicochemical meat quality, although slight deviations from the control (no soy substitution) were observed. Broiler chickens fed SP produced a meat colour with more intense red and yellow hues than the control. Additionally, in the first broiler study the SP-samples had a higher ultimate pH, which likely resulted in their observed higher water-holding capacity; this finding was not replicated in the second study. In the second study the effect of industrial (highly-oxygenated modified atmosphere) packaging in connection with feed was also tested; surprisingly, SP feed resulted in increased lipid oxidation. SP and HI had little influence on meat quality in pork production; however, HI appeared more versatile in substituting soybean meal than SP, as meat produced with HI exhibited smaller deviations from the control product. Additionally, both broilers and barrows

tended to be heavier when fed HI, as well as had higher amounts of saturated fatty acids; particularly C12:0 and C14:0.

Eating quality was evaluated by a trained sensory panel using a sensory profiling method. Sensory analysis pointed to only minimal differences derived from protein feed. In broiler production, panelists described SP-fed chicken breast as having less intense off-attributes (barn odour and metallic flavour) and increased umami and chicken flavour; however, the differences were small compared to the control and difficult to replicate. Pork chops produced with SP were described as minimally more astringent. HI resulted in more consistent findings across multiple studies and meat products. Overall, HI led to a more intensive odour and flavour, and pork chops produced with HI were described as juicier.

Finally, a discrete choice experiment was conducted to evaluate consumer acceptance of the unfamiliar chicken breast colour resulting from SP feed and observe the consumer preference for chicken breast produced with insect feed. The experiment was part of an online survey, disseminated via a commercial consumer panel. The resulting random parameter (mixed-) logit model suggests that German consumers would reject chicken breast produced with SP, on average; however, consumers would be more accepting when supplied with information about the origin of the product's atypical colour. Nonetheless, they had no significant preference for the SP-fed product above the standard product. Consumers prefer chicken breasts produced with insect feed over the standard soy-fed product. Furthermore, when consumers are informed concerning feed type in production, the intensity of preferences (willingness-to-pay in preference space) increases. However, it is important to note that consumers who are not motivated by sustainability strongly reject chicken breast labelled as "fed with insect meal". This shows that while feed labelling and information can help overcome consumer hesitation in one aspect (abnormal colour), it can also undermine consumer confidence in another (disgust factor). Nevertheless, the labelling of feed in meat production was recommended in order to ensure overall economic benefits and to allow consumers to make choices according to their own values and preferences.

Overall, this research demonstrates that SP and HI can be incorporated into poultry and pork diets without compromising meat quality or consumer acceptability. Additionally, information regarding the feed used during production should be provided to consumers to support consumer preference for these alternative products.

Table of Contents

Project: Sustainability Transitions in the Food Sector

Sustainability Transitions

Food sectors are comprised of multiple social and technical elements; they are often a complex socio-technical system that incorporates various institutions (at multiple jurisdictive levels), varying firm size and structure, technology and infrastructure, personal identity and culture, markets, and natural resources (Geels, 2005; Markard, Raven, & Truffer, 2012). Such a complex network-like structure can appear sporadic and unsystematic. Therefore, Geels (2005) proposed a multi-level perspective to better describe and understand how socio-technical systems innovate. The multi-level perspective is based on evolutionary economics, sociology of technology, history of technology and innovation sciences and identifies three levels used to understand and analyse systems (Geels, 2005). The micro-level consists of small protected niches where drastic innovation takes place. The meso-level is where the socio-technical regimes exist, as social and technical elements are interdependent and interact. Finally, the macro-level is the vast 'landscape' where relatively little interaction occurs as actors have relatively little influence over the structure and direction (Geels, 2005).

By applying the multi-level perspective to sectors focused on sustainability-motivated innovation, the body of sustainability transition literature was born (Markard et al., 2012). Sustainability transition studies apply the technological innovation systems framework, which is similar to the multi-level perspective except it applies a heavier institutional and public policy focus on strategies to foster innovation (Coenen & Truffer, 2012). Overall, sustainability transitions have been defined as fundamental, long-term, multi-dimensional shifts in socio-technical systems, where the system inherently changes to encompassing more sustainable modes of production and consumption (Markard et al., 2012). Often system transitions are mired by strong path-dependencies and 'lock-ins', such as user habits, established technologies and infrastructure, as well as institutional, political, and legal structures (Markard et al., 2012). Barriers to system transformation should be identified and removed wherever possible. In addition, niches should be nurtured as protected areas to foster innovation. This is fundamental to sustainability transitions; even though not all niches successfully evolve into regimes (Smith, Voß, & Grin, 2010).

Despite the incredible amount of literature outlining the underpinnings of the multi-level perspective and its application in sustainability transitions, there still remain multiple holes in the framework, and more validating evidence based on real data is required in multiple sectors. First, the theory is largely abstract and not geographically-centered; more research into how geography and space effect sustainability transitions is needed (Coenen & Truffer, 2012). In addition, hierarchies are largely left out of the framework; their inclusion could provide additional understanding to complex socio-technical systems (Markard et al., 2012). Finally, despite having sustainability in its name, there is no discrete mechanism to assess the degree of innovation in terms of overall sustainability gains. In other words, studies often remain at the abstract level, assuming that a transition from 'A' to 'B' is more sustainable based on normative assumptions. Incorporating a sustainability index or measure into studies could clear up misconceptions as to whether a transformation is actually safeguarding social, economic, and environmental aspects of the system. Such an element could help provide explanations behind the differences in transformation process and success. As a cross-country study driven by the European protein gap, the project 'Sustainability Transitions in food production: alternative protein sources in socio-technical perspective' aims to provide context specific findings in order to overcome such limitations apparent in Sustainability Transitions research.

Project objectives

'Sustainability Transitions in food production: alternative protein sources in socio-technical perspective' funded by the *Niedersächsisches Vorab* from the Ministry for Science and Culture, Lower Saxony, Germany applies the sustainability transitions concept to animal production in three geographical regions. The project's eight working groups study the entire animal production value chain, focusing on pork and poultry production - from feed suppliers to meat consumers - in order to identify where system path-dependencies and/or 'lock-ins' may hinder a food system transformation. The food system transformation in question is whether substituting soybean meal imports with two promising alternative protein sources – spirulina (*Arthrospira platensis*) and black soldier fly (*Hermetia illucens*) larval meal – would be sustainable and accepted along the value chain. Spirulina is a microalga (cyanobacteria; blue-green alga) that has been highly researched and is highly commercialized in East Asia; although it is more commonly sold world-wide as an anthropogenic dietary supplement (Chen et al., 2016). Black soldier fly larvae have more recently been semi-commercialized; the larval meal used in the experiments of this dissertation were sourced in Germany. Both alternative protein sources have been selected based on previous research highlighting their high protein contents and ability to be produced independent of arable land; therefore, making them potentially more sustainable than soybean meal imports originating from South America.

Objectives of this thesis

The author was tasked with evaluating the resulting physicochemical and organoleptic (eating) meat quality acquired from swine and broilers fed spirulina or black soldier fly larval meal. Ascertaining the resulting meat quality is important to identify potential path-dependencies and 'lock-ins'. For example, if the resulting meat quality characteristics remain unchanged to the current conventional product, one would assume that path-dependencies at the packer and processing level become less relevant as no specific change resulting from the transformation is observed at the primary level. In addition, consumer level perceptions and preferences are monitored to estimate consumer acceptance and marketing potential.

Using a wide array of methods, this dissertation aims to assess the meat quality of alternatively-fed swine and broilers in comparison to conventional soybean meal-fed animals. Research papers 1-3 included in this dissertation focus on monitoring and evaluating the fundamental physicochemical meat quality aspects for the two most popular cuts of meat in Germany – pork loin and chicken breast. In addition, sensory profiling was carried out for both products. Finally, research paper 4 includes an online discrete choice experiment using real-life photos in order to estimate consumer preferences of chicken breast produced with spirulina or black soldier fly larval meal (in the study referred to as insect meal). The study also investigates the effect of feed identification and information regarding the alternative protein feeds.

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The Soybean Dilemma

This chapter discusses the establishment of soybean meal as one of the most promising, yet debated, agricultural commodities in the animal production sector. For brevity, this chapter is limited to the Western perspective(s). However, it needs to be acknowledged that soybeans have been cultivated for millennia in China and many centuries in other Asian societies and as a result soybeans hold another importance in these regions (Hymowitz, 2008).

The miracle protein feed – soybean meal

Currently, soybean (*Glycine max* (L) Merr.) meal is the most widely fed protein source in monogastric animal production systems, such as pork and poultry. Both of these systems desire and – for efficiency – require energy-rich feeds high in carbohydrates and protein (Schedle, 2016). Both components of which are provided by soybean meal (Sotak-Peper, Gonzalez-Vega, & Stein, 2015), making it a very desirable feed from an animal nutritional stand-point. However, like every established custom, this development did not occur suddenly, nor of its own accord.

Modern research has primarily focused on improving the nutritional quality of soybeans through breeding (Clarke & Wiseman, 2000; Cromwell, Monegue, Randolph, Coffey, & Stahly, 2017), post-harvest processing measures (Mukherjee, Chakraborty, & Dutta, 2016), as well as animal diet formulation and supplementation (Denbow, Ravindran, Kornegay, Yi, & Hulet, 1992). Soybeans were not initially perfectly suited for their role in an anthropogenic animal production system; they contain multiple anti-nutritive substances, such as trypsin inhibitors, lectins, and oligosaccharides (Stein, Lagos, & Casas, 2016). However, after decades of research their properties in monogastric animal diets are relatively well-understood, making them well-appreciated by the animal feed industries (Willis, 2003).

Soybean meal nutritional components

Soybeans are primarily produced for their oil (Hymowitz, 2008). Nonetheless, once the oil has been extracted, the leftover meal (soybean meal) is a high-value, marketable co-product. This co-product is used in animal nutrition because it is high in crude protein and essential amino acids (Baker, Liu, & Stein, 2014). Although the nutritional composition can differ based on region of cultivation, only small adjustments to diet formulation are required (García-Rebollar et al., 2016). There are two types of soybean meal available for use in swine and poultry diets:

high-protein and low-protein soybean meals. High-protein soybean meal is produced from dehulled and defatted soybeans and has a crude protein content of ~48% (Stein et al., 2016). Low-protein soybean meal is produced from non-dehulled soybeans resulting in a crude protein content of ~ 43% (Stein et al., 2016).

Soybean meal is entrenched as the main protein source in animal feeds. Soybean meal contains crucial amino acids, such as lysine and tryptophan, in high amounts which are particularly important for pork and poultry production (Beski, Swick, & Iji, 2015; Stein et al., 2016). The high levels of essential amino acids allow soybean meal to complement other plant-based ingredients, resulting in a well-balanced amino acid feed composition (Stein et al., 2016). In fact, it has been and continues to be, the standard protein source (control) against which the quality of alternative protein feeds are measured (Leeson, Atteh, & Summers, 1987; Navarro, Mathai, Jaworski, & Stein, 2018; Stein et al., 2016).

Congruently, standardized ileal digestibility of amino acids is also an important indicator for protein quality. Standardized ileal digestibility is an estimation of the percentage of digested amino acids from different feed ingredients (Stein et al., 2007). Amino acid content in the feed is of high importance, but standardized ileal digestibility is just as important because it estimates whether the amino acids can be digested and therefore utilized by the animal. In this respect, soybean meal has a superior standardized ileal digestibility compared to numerous other plant-based feed ingredients (González-Vega & Stein, 2012).

Research achievements to improve nutritional quality

Despite its advantages, soybean meal also contains anti-nutritive compounds (Beski et al., 2015; Stein et al., 2016); however by circumventing many of anti-nutritive compounds found in soybeans, improvements in soybean meal quality have been established. For example, it was identified early on that trypsin inhibitors, which suppress enzymatic components of digestion due to the enzyme trypsin, can be effectively denatured though heat treatment (Stein et al., 2016; Waldroup, Ramsey, Hellwig, & Smith, 1985). Soybean meal is also a good source of phosphorus; however it is bound to phytate (Stein et al., 2016). One technique to increase the bioavailability of phosphorus is by supplementing the feed with microbial phytase (Almeida & Stein, 2010); another is through fermentation (Rojas & Stein, 2012), or treating soybean meal with enzymes (Rojas & Stein, 2017). These technologies allow soybean meal to replace animal protein sources, such as fish or poultry by-product meals (Stein et al., 2016) in monogastric animal diets. Fermentation and enzyme treatment of soybean meal have also been shown to reduce antigens and oligosaccharides, which otherwise can negatively affect gastro-intestinal health (Stein et al., 2016). In poultry production, another approach to circumvent the anti-nutritive substances in soybean meal is to incorporate highly processed soybean protein concentrates or isolates into diets (Beski et al., 2015); however this is costly. Finally, new varieties of soybeans have been bred to contain lower levels of oligosaccharides or even higher concentrations of protein (Baker et al., 2014), allowing soybean meal to be more effectively incorporated into swine and poultry diets.

Aside from reducing anti-nutritive substances, other improvements in soybean meal include the breeding of genetically modified (GM) soybean varieties with higher yields, to increase and ensure soybean supply for animal feed. So far, studies with both swine and poultry have shown that GM soybean meal is equivalent to that of non-GM (conventional) soybean meal (Flachowsky, Chesson, & Aulrich, 2005).

Conclusion

In summary, soybean meal is a readily available co-product of soybean oil production. Inherently, soybean meal is an advantageous plant-based feed ingredient due to its attractive nutritional content that can contribute high amounts of crude protein and energy to an animal's diet. Nonetheless, decades of research have been invested into improving its quality for the use in swine and poultry diets, enhancing its superiority to become the most used protein feed in animal production.

The global game of soybean production

Due to its desirable applications in animal nutrition, bio-energy, food (technology) and industrial sectors, the demand for soybeans has sky-rocketed. Subsequently, production is not forecasted to plateau any time soon (Goldsmith, 2008). Although historically Western production was predominantly centered in the USA, with exports being sent to Europe, (Martin, 2014); nowadays, Brazil and Argentina are the second and third most important exporters (Masuda & Goldsmith, 2009) and supply the majority of soybeans for the European market (Martin, 2015). In addition, European preferences have changed to increase demand for non-GM soybeans (Martin, 2015), which has had multiple implications for land use and market segregation in soybean producing regions.

The early history of soybean production

Most records point to the domestication of soybeans in China some millennia ago; a number of European explorers and clergy documented the Asian cultivation of soybeans and their uses (Hymowitz, 2008). However, it was not until the late 18th and 19th centuries that the crop received significant attention outside of Asia. In Western Europe (France, England and the Netherlands) the seeds of soybeans were primarily grown in in botanical gardens for taxonomic purposes; yet there are records of soybeans being used in Croatia and Serbia as a dietary supplement to increase poultry system production as early as the late 17th century (Hymowitz, 2008).

However, the largest contribution to the Western utilization of soybeans is due to private and government-funded research in the USA. Although not the first recorded person to cultivate soybeans in North America (Hymowitz & Harlan, 1983), Dr. Mease is known for coining the term 'soybean' in English and released an early research report on the crop in 1804 (Hymowitz, 2008). Nearly 100 years later, the United States Department of Agriculture (USDA) catalogued their first soybean seeds and employed William J. Morse, who spent his career fostering the production of soybean nation-wide (Hymowitz, 2008). A decade later, it was observed that the pre-treatment of heating improved the nutritional value of soybean meal for use in animal diets (Osborne & Mendel, 1917) and private and public breeding initiatives were steadily supported (Hymowitz, 2008), contributing to the USA becoming the world's largest soybean producer by the 1950s (Hymowitz, 1970). A title that the country continues to defend (FAOSTAT, 2017).

South America's evolution as a supplier

Although the USA remains the top producer globally, it is also a heavy-user of soybeans, incorporating them as an input component in multiple supply chains, such as biodiesel, industrial products, and animal feed and food processing (Goldsmith, 2008). The story is much different in South America where soybeans are produced almost exclusively for exports; 70% of Brazil's total production is exported (Richards, Myers, Swinton, & Walker, 2012).

In the early 1990s, political change and a liberalization of markets allowed Brazilian farmers to produce for world markets in a time when global demand for protein sources was growing (Richards et al., 2012). The increase in demand was initially driven by Europe (Richards et al., 2012); however today China is the largest importer of soybeans (Martin, 2014; Song, Marchant, Reed, & Xu, 2009). Free markets and increasing global demand coincided with a devaluation in South American currencies compared to the US dollar, making soybeans lucrative for South

American farmers, who had a comparative advantage compared to the USA, whose farmers did not benefit from their high value currency (Richards et al., 2012). This drove a rapid increase in production through the expansion of cultivated area by substituting out other crops, utilizing pasture areas, and deforestation from the 1990s until 2007 (Masuda & Goldsmith, 2009). Deforestation was brought under control through a governmental action plan and other domestic and international interventions starting in 2004 (Boucher, Roquemore, & Fitzhugh, 2013), and soybean production no longer directly nor indirectly influences land conversion (Richards, Walker, & Arima, 2014). Nonetheless, European demand is still influencing the structure of soybean supply and land use allocation for soybean cultivation (Garrett, Rueda, & Lambin, 2013).

The European protein gap

Europe only produces a negligible quantity of soybeans. For years, agricultural policy in Europe undermined the cultivation of oilseed crops in exchange for cereals, so that total arable land area for protein cultivation has decreased since the 1960s despite an increase in demand for these crops (Martin, 2014). Current European animal production relies on imports for 95% of its soybean requirements and over 64% of animal feed protein comes from soybean meal (de Visser, Schreuder, & Stoddard, 2014). The remainder of protein in animal feeds is derived from the incorporation of cereals into animal diets (Martin, 2014). Extraordinarily, Western Europe produces a surplus of animal products, which further exacerbates the scale of protein imports required; poultry stock is positively correlated with soybean imports in the EU (Boerema et al., 2016). Overall, this regional imbalance between protein feed required and protein feed supplied is known as the European 'protein deficit' or 'protein gap.'

The protein gap has started to receive negative attention in some European countries, despite the fact that soybeans are relatively invisible to the average consumer given that demand for soybeans originates from the demand for animal products (Goldsmith, 2008). With the rise of the GM soybean, geopolitical lines were drawn between producer and consumer countries based on the acceptance of GM soybeans (Young, 2011), with the precautionary principle countering its popularity in Europe. The result is that the European Union (EU) strictly regulates the cultivation of GMOs and their incorporation into the food supply (Vigani & Olper, 2013).

Changing demands – non-genetically modified soybeans

Notwithstanding strict standards, GM feed concentrates constitute approximately 85% of animal feed supply in the EU (Martin, 2015). This is likely because, animal products produced using GM soybean meal do not have to be declared, unlike other foods produced with GMOs (Venus, Drabik, & Wesseler, 2018). Consumers have become increasingly aware of the non-transparent labelling of animal products and claim to prefer animal products produced with non-GM feed. This is the case in Germany (Profeta & Hamm, 2018) and has led numerous meat, egg and dairy producers to voluntarily label products as 'without GMO.' However, 75% and 100% of broiler production in Germany and Austria, respectively, is considered non-GM (Martin, 2015). It is possible to substitute soybean meal by other ingredients in pork and dairy diets. Yet, soybean meal continues to be an important dietary component in broiler production, as explained previously. In addition, the proportion of non-GM feed used in animal production continues to increase across multiple animal product sectors and multiple EU countries (Martin, 2015). Subsequently, consumer-side niching and strong political-will to enforce approval processes (Venus et al., 2018) has resulted in a segmented soybean sector, primarily in Brazil, where the Brazil's province of Mato Grosso is the leading producer for non-GM soybeans (Garrett et al., 2013).

From the producer side, the cultivation of non-GM soybeans presents an added level of security because of the strong quality preference driving demand for this crop compared to GM soybeans (Garrett et al., 2013). Non-GM soybean producers are more insulated against the increasing value of domestic currency. Typically, as explained by Richards et al. (2012), this would result in decreased competitiveness; however, Garrett et al. (2013) illustrate that non-GM soybean production and export quantities destined for European markets increased in Brazil during times of increasing currency value in Mato Grosso. Therefore, despite comprising a relatively small portion of worldwide soybean production, European demand for non-GM soybeans substantially influences land allocation decisions regarding soybean cultivation in Brazil (Garrett et al., 2013).

In addition, as a result of this guaranteed market with high quality expectations, non-GM soybeans also fetch a premium price, between 16 and 58 USD per ton depending on world market soybean prices and certification (Garrett et al., 2013). Price premiums depend on certification schemes; the fundamental CERT-ID warrants the majority of the price premium between 16 to 54 USD, as recorded over a three-year period, and ProTerra or a Roundtable on Responsible Soybeans (RTRS) certification additionally contribute approximately 4 USD or

1.50 USD per ton, respectively (Garrett et al., 2013). The differences in price premiums are a result of the various certification scheme requirements and structures. The ProTerra and RTRS schemes include environmental damage prevention measures. Both necessitate that cultivation does not take place on newly converted land. ProTerra does not allow certification if land used for cultivation was converted after 2004; RTRS's cut-off for land conversion is 2009 (Garrett et al., 2013). Necessary compliance with these restrictions for price premiums is what partially segregates the agricultural land markets in Brazil.

Environmental governance and commercialization in South America

The added layer of environmental governance, along with reduced pesticide use, has created the perception that non-GM agricultural production benchmarks as more sustainable than its GM counterpart; however, this is only the case in some regions of Brazil (Gaitán-Cremaschi, Kamali, van Evert, Meuwissen, & Oude Lansink, 2015). Furthermore, organic soybean production, although considered the most sustainable form of production, has been linked to higher land occupation compared to both GM and non-GM production systems (Pashaei Kamali et al., 2017). As a result of certification standards linked to the year of land conversion, diverse land price markets based on current and past land use have emerged (Richards et al., 2014). For example, in 2010 forest land prices were nearly 100 times lower than cropland; this appreciation in crop land values is suspected to be contributing to regional forest loss (Richards et al., 2014). Furthermore, Gasparri & le Polain de Waroux (2015) estimate that as long as conservation efforts are based on production units, such as fields and states, leakages in policy still exist to enable deforestation. These factors combined with heterogenous management and supply chain factors, such as access to infrastructure, explain why the sustainability of soybean production is highly system- and region-specific (Gaitán-Cremaschi et al., 2015; Prudêncio da Silva, van der Werf, Spies, & Soares, 2010).

Overall, although far from perfect, soybean production in South America should be considered a success story. The effective environmental governance structures implemented in Brazil have stagnated deforestation (Boucher et al., 2013). In addition, technological development pulls family farms away from subsistence living, as is currently taking place in Bolivia, Colombia and Peru (Barrientos-Fuentes & Torrico-Albino, 2014). With decreasing individual family farm land sizes and aging rural populations, farms consolidate and specialize (Barrientos-Fuentes & Torrico-Albino, 2014), a logical transition to maintain and increase agricultural production levels. In general, the agricultural sector becomes monetized with the outcomes of improved market integration, increased diversity in incomes, and food security becomes income dependent rather than self-sufficient (Barrientos-Fuentes & Torrico-Albino, 2014). Sometimes these changes take place equitably. For example, indigenous communities involved in agriculture are able to profit from the monetization of the agricultural sector by securing modern living conditions and opportunities for the benefit of their members (Barrientos-Fuentes & Torrico-Albino, 2014). A once predominantly poor and isolated region of Brazil, incomes in the Brazilian Amazon have reached the national average, and the socio-economic growth in the region has appeared to also stabilize deforestation (Tritsch & Arvor, 2016). In other words, gaining access to monetized markets can give rural citizens increasing flexibility in choice of livelihood and stabilize potential environmental damages after a point where basic needs are met.

However, as is too often the case, commercialization can result in large income disparities and conflicting land use valuation (Steward, 2007). In addition, environmental concerns like nutrient cycling – or lack thereof – related to soybean production in South America (Lassaletta et al., 2014; Lathuillière, Johnson, Galford, & Couto, 2014); virtual water exports (V. de P. R. da Silva et al., 2016; Lathuillière et al., 2014); and indigenous rights and access to land (Steward, 2007) are all problems that still remain to be addressed in specific regions.

Irreconcilable interests

Despite the large environmental and socio-economic improvements that the soybean sector has seen since 2004, environmental issues, as in any agricultural sector, still remain in Brazil. It is this imperfection which informs the normative position in Europe vilifying soybean production in South America, i.e., that soybean imports should be avoided, and Europe should become self-sufficient. Martin (2015) also argues that working towards protein self-sufficiency could stimulate local economies; especially if animal product marketing can capitalize on the consumer preference for local non-GMO feed (Profeta & Hamm, 2018). However, there is limited knowledge on whether local production is more sustainable than reliance on South American imports, especially given that environmental impacts in Brazil are related to production intensification rather than land changes (Garrett & Rausch, 2016). Soybean production in Europe is of an intensive nature as well. In addition, the regulations on cultivating GM crops in EU countries have been reconciled with world trade markets so that domestic production no longer rules out products being non-GMO (Young, 2011). Therefore, it remains unlikely that self-sufficiency would resolve normative concerns posed by European consumers.

Europe's demand for protein feed (de Visser et al., 2014) coupled with its lack of current competitive advantage and decreasing production (McFarlane & O'Connor, 2014) means that without an agricultural system overhaul, Europe will remain reliant on imports (Martin, 2015). In addition, Brazil's adaptive and receptive soybean production and specialization in non-GMO soybean production has shown that they are willing to satisfy one of their largest customer-bases, and these changes can translate into sustainability gains (Garrett et al., 2013). Therefore, de-coupling the European import market from South American exporters remains futile as soybean international trade continues to expand (McFarlane & O'Connor, 2014).

Seeing past the dilemma

Due to centuries of research in multiple agricultural fields, soybean meal is the most well-known and widely-used animal feed protein source. Its advantageous amino acid composition coupled with easily implemented processing steps to reduce anti-nutritive substances is what drives the demand from the animal production sector for this nearly ideal protein feed. In addition, the cultivation of soybeans is well-understood, and genetic research has led to unique varieties, some of which are considered GMOs, so that soybean cultivation has expanded drastically over the last 70 years.

This expansion proved decidedly advantageous for South American countries who were able to capitalize on the demand for soybeans because of low-value national currencies, compared to other supplying and demanding countries, in the late 1900s. South America became the top global supplier of soybeans and therefore soybean meal. Regions with limited arable land for protein production, but a high consumption of animal products (i.e., Europe), became dependent on soybean meal imports to meet domestic needs of their dairy, meat and egg production. This is known as the European protein gap and was largely domestically ignored, while relationships between global soybean stakeholders were harmonious. However, with the sudden expansion of soybean cultivation and the commercialization of agriculture in many regions of South America, came increasing European consumer concerns: their first concern was consuming GMOs; their second was Amazon rainforest deforestation. Regions of Brazil specialized in non-GM soybean production to placate consumers and the government implemented non-deforestation policies to curb environmental damages. This has led to a dilemma spanning the Atlantic Ocean in a search for a morally responsible production system without compromising the quantity of animal protein consumed by European consumers.

Regardless of the sustainability of soybean imports, Europe has a food security dispute on its hands. In 2006, the EU was surpassed as the number one importer of Brazilian soybeans by China (Lathuillière et al., 2014); China has strong market power in determining global soybean supplies (Song et al., 2009). Furthermore, animal-based protein consumption is increasing worldwide (FAO, 2015) and in order to meet future demand, new protein sources will need to be exploited regardless of regional (or continental) trade flows (Röös et al., 2017).

In short, the EU's concerns regarding their protein deficit are more than justified, but largely for the wrong reasons. Soybean production abroad is not the villain that it is made out to be; rather, the required global protein production needs to be diversified and augmented. In order to secure stable access to protein feeds needed for a strong animal production sector, the EU should research protein sources independent of arable land, which also require few inputs. Two such protein sources are the topic of the research papers in this dissertation. Just as with soybeans, long-term research investments will be required to identify, improve, and integrate alternative animal protein feedstuffs into becoming the norm.

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Fundamental Aspects of Meat Quality

‘Meat quality’ broadly denotes the *ante* and *post mortem* preparation of animals. Yet, meat quality encompasses numerous factors from farm to mouth (Toldra & Flores, 2004). It includes microbiological and toxicological concerns, observable physicochemical parameters, nutritive and sensory attributes, and even ‘wholesomeness’ (Sundrum, 2010). To complicate things further, the traits and thresholds of what is considered ‘good quality’ differ between products, regions (Oliver et al., 2006; Grunert, 1997), and contexts (Korzen & Lassen, 2010). Therefore, to objectively examine aspects of meat quality, ‘quality meat’ needs to first be defined. This chapter describes three components of quality and their importance in discerning ‘meat quality;’ specifically, physicochemical quality, eating quality, and consumer perceived quality according to the Total Food Quality Model (Grunert, Bredahl, & Brunsø, 2004) will be addressed.

Physicochemical quality

Physicochemical parameters are most used to evaluate meat quality. These parameters are objectively measurable and often closely associated with one another; they are physical and/or chemical compounds or states assessed in a laboratory setting as indicators for meat quality. This section, which is not a comprehensive list, outlines a few of the fundamental parameters used for determining physicochemical meat quality: meat colour, pH, water-holding capacity, instrumental tenderness, and lipid oxidation. Understanding the parameters mentioned here is essential to discussing the outcomes of the research papers submitted as the main body of this dissertation. In addition, the case of pale, soft, exudative (PSE) meat, is described to illustrate how physiochemical meat quality parameters are interconnected.

Meat colour

Meat colour is often the first indicator of quality assessed by consumers (Kennedy, Stewart-Knox, Mitchell, & Thurnham, 2004). However, a variety of factors contribute to meat colour. Most commonly, meat colour is associated with the oxidation and reduction of heme pigments, i.e., myoglobin and hemoglobin (Fox, 1966) due to the biochemical reaction between these pigments and oxygen. Secondarily, carotenoid deposition in muscle tissues, which is influenced by dietary carotenoid-intake (Hatlen, Jobling, & Bjerkeng, 1998), can also influence meat colour.

Usually, meat colour refers to the presenting colour of cut meat as a result of the biochemical reaction between heme pigments and oxygen. Because animals are bled during slaughter, hemoglobin plays a minimal role in influencing meat colour (Fox, 1966). Therefore, the focus remains on myoglobin. The reaction between myoglobin and oxygen is not unidirectional, but rather is a dynamic relationship involving myoglobin, oxymyoglobin, and metmyoglobin. Bright red carboxymyoglobin is also relevant in light of increasing interest in carbon monoxide (CO) modified atmosphere packaging (Mancini & Hunt, 2005). However, as CO modified atmosphere packaging is currently prohibited in the German market (Grebitus, Jensen, Roosen, & Sebranek, 2013), only the first three compounds will be discussed here.

In an anaerobic atmosphere, such as prior to cutting a carcass, myoglobin is initially purple; when exposed to air, it takes on oxygen to form the bright red oxymyoglobin (Figure 1). This red state is stable so long as the myoglobin remains in an oxygen-rich atmosphere (Fox, 1966). Simultaneous with the production of oxymyoglobin, heme pigments oxidize to form metmyoglobin, which confers a brown colour; this is particularly relevant in low oxygen atmospheres (Fox, 1966), such as in sublayers underneath the surface of a freshly cut piece of meat (Mancini & Hunt, 2005). These reactions are bidirectional and therefore metmyoglobin can reduce back to oxymyoglobin when placed in a high oxygen atmosphere (Fox, 1966).

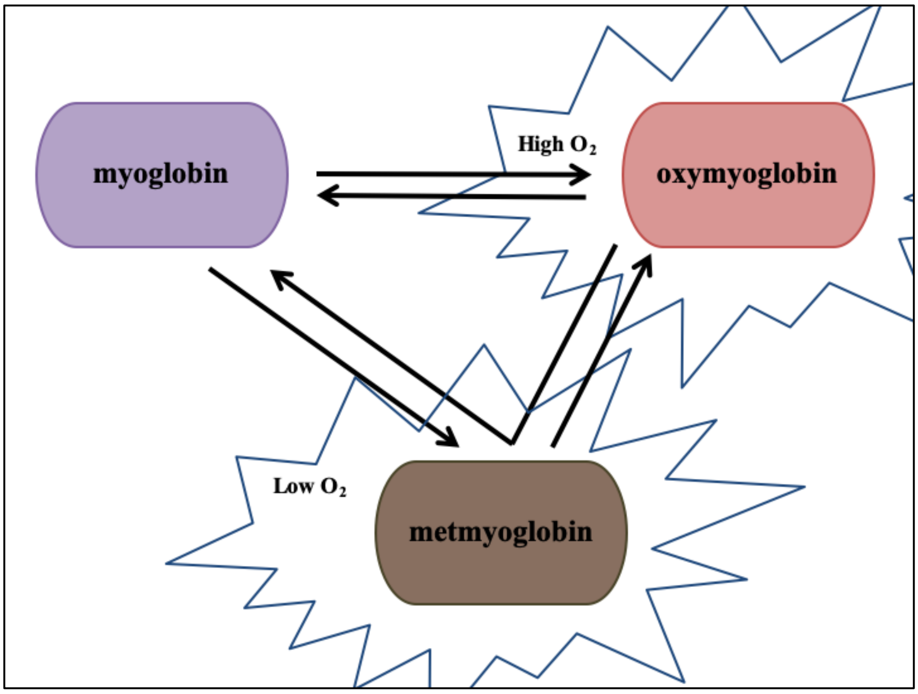

Figure 1: Illustration of interaction between heme pigments in meat colour development (based on Mancini & Hunt, 2005)

Colour formation through myoglobin chemistry is the same for all meat types. However, intensity of colour is dependent on myoglobin content (Kim et al., 2012), which is linked to: muscle type and histochemical structure (James, 1968; Kranen et al., 1999); livestock and poultry type and genetics (Mancini & Hunt, 2005); as well as physiological stage (Wideman, O'Bryan, & Crandall, 2016). The rate and degree of colour formation is influenced by atmosphere oxygen concentrations; an appealing bright red colour can be reliably maintained for extended periods in a highly oxygenated atmosphere before the formation of metmyoglobin discolours fresh meat products (Mancini & Hunt, 2005). Such a colour is maintained with the use of highly-oxygenated modified atmosphere packaging (HiOx MAP). In this regard, myoglobin chemistry is considered the most important process for influencing redness (a*) instrumental values in meat.

Although more commonly associated with salmonid production, carotenoid deposition in muscle tissues influences meat colour. The ability to determine meat colour through dietary supplementation is appealing to animal producers and therefore the influence of carotenoids in livestock and poultry systems has also been investigated and shown to effect meat and egg yolk colour (Holman & Malau-Aduli, 2013). Feeds high in carotenoids have been found to decrease lightness (L*) instrumental values (Altmann, Neumann, Velten, Liebert, & Mörlein, 2018; Hatlen et al., 1998), and increase redness (a*) values as well as yellowness (b*) values (Hatlen et al., 1998; Toyomizu, Sato, Taroda, Kato, & Akiba, 2001) when fed at high concentrations. As is captured in Papers 1, 2 & 4, increasing a* and b* values through carotenoid deposition may not equate to improved meat quality, as is often assumed to be the case with heme pigment

In pork and broiler production, L* instrumental values are also of great importance. High L* values coincide with cases of PSE meat (Barbut et al., 2008). Lower than average lightness (L*) values for pork can also help identify dark, firm, dry (DFD) meat (Brewer, Zhu, Bidner, Meisinger, & McKeith, 2001). The connections between meat colour and PSE meat will be discussed in section *the case of pale, soft, exudative (PSE) meat*.

Finally, meat colour is usually modelled in Commission on Illumination (CIE) L*a*b* colour space using a camera-like chromameter (colourimeter); it can also be visually assessed using standardized colour cards (M. Font-i-Furnols, Candek-Potokar, Maltin, & Prevolnik Povše, 2015). In addition, computer vision and digital imaging automatically and expediently determine meat colour on the slaughter line (Mancini & Hunt, 2005). As colour measurements are largely non-destructive and quick to perform, meat colour is an ideal first indicator for identifying possible cases of compromised meat quality, such as PSE or DFD meat. However, in order to do this accurately, it is necessary to first identify and evaluate the colour formation pathway, heme pigment oxidation vs. carotenoid deposition, so as to correctly infer meat quality implications based on colour measurements.

pH

An important indicator for the rate of conversion from muscle to meat, pH is simple to assess on the slaughter line. It is also highly connected to other physicochemical quality parameters associated with processing and eating quality, such as water-holding capacity and tenderness (Popp, Wicke, Klein, & Krischek, 2014). At homeostasis, a muscle has a pH of ~7 and muscle tissue converts glycogen (glucose) into pyruvic acid to regenerate ATP (Adenosine triphosphate) stores through the process of glycogenolysis (glycolysis). However, *post mortem* muscle metabolism occurs anaerobically so that glycogen is converted to lactate, reducing the pH. How quickly and how far pH decreases depends on muscle glycogen stores (Onopiuk, Półtorak, & Wierzbicka, 2016) and the rate of ATP degradation in the muscle, which regulates glycogenolysis and glycolysis, post-slaughter (Krischek, Natter, Wigger, & Wicke, 2011).

The type of muscle fiber, genetics of the animal, and pre-slaughter-handling are all key components affecting meat pH. Muscle fibers are categorized as 'fast white', 'intermediate' or 'slow red.' Although 'white' and 'red refer to heme pigment concentrations, 'fast', 'intermediate', and 'slow' refer to glycolytic potential (high, intermediate, low) describing muscle metabolism (Monin, Mejenes-Quijano, Talmant, & Sellier, 1987). The homeostatic rate of muscle metabolism in each muscle type affects pH decline *post mortem*, so that 'fast' muscles have a faster average rate of pH decline than 'slow' or 'intermediate' muscles (Popp et al., 2014). Besides muscle type, genetic factors, such as breed and individual metabolism regulation, also influence glycolytic potential (Monin et al., 1987) and ATP degradation post-slaughter (Krischek et al., 2011) and therefore pH. The RN gene has been identified in swine; gene-carriers have above average glycogen stores which results in a below normal pH decline *post mortem* (Scheffler & Gerrard, 2007). In addition, resilience to stress is also often associated with genetically-linked factors, such as breed (Adzitey & Nurul, 2011) and the presence of the halothane gene in pigs (Scheffler & Gerrard, 2007). Finally, pre-slaughter animal handling has a strong effect on meat pH, as any animal under stress experiences a rush of adrenaline, resulting in a high rate of ATP degradation to fuel the evolutionary 'fight or flight' response, thus stimulating glycogenolysis and glycolysis. If the rate of metabolism remains high, the decline in pH is more rapid than normal (Figure 2). If glycogen stores are nearly consumed *ante mortem*, it can result in an above normal pH due to low lactate production (DFD meat).

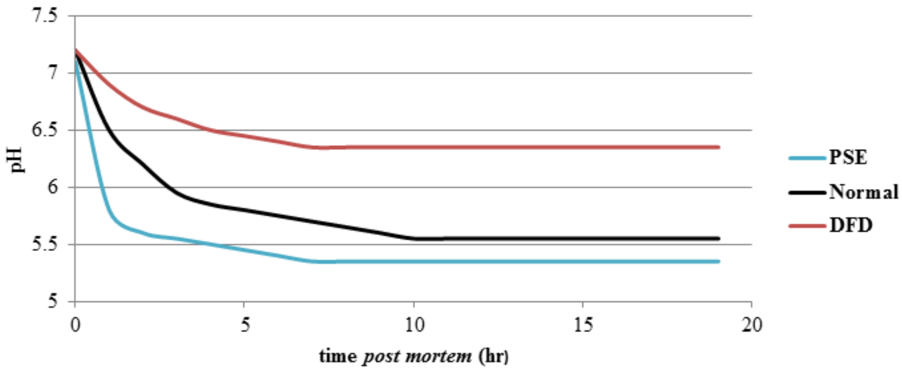

Figure 2: Illustration of pH decline *post mortem* in pork (based on Font-i-Furnols et al., 2015)

Although numerous factors affect pH and the rate of decline is more telling than the absolute decline, a range of acceptable ultimate pH values have been recommended for different meat types. For example, suggested ultimate pH values for beef and pork (loin) are between 5.5 and 5.8; whereas chicken (breast) meat values should be between 5.8 and 6.0 (Font-i-Furnols et al., 2015), muscle dependent.

Water-holding capacity

Water-holding capacity is of particular economic importance, because meat is composed of over 70% moisture and sold by weight. Numerous biochemical, fiber structural, thermal and mechanical factors affect water-holding capacity, and there are multiple parameters used to determine water-holding capacity of meat.

Water-holding capacity is connected to both pH (decline) and tissue structure (Bowker & Zhuang, 2015). In a homeostatic state, the proteins actin and myosin interact to form a filament net that retains moisture within the meat (Huff-Lonergan, 2006). However, initial pH decline *post mortem* leads to denaturation and an eventual degradation of these proteins, resulting in a reduced ability to maintain moisture (Bowker & Zhuang, 2015; Huff-Lonergan & Lonergan, 2005). The moisture that leaks from the actin-myosin matrix then accumulates between fiber bundles to be released through cutting or other muscle structure disruptions (Honikel, 1998). The rate of moisture loss is influenced by factors such as: cutting and its orientation to fibers, rate of temperature decline after slaughter, and temperature during storage (Huff-Lonergan, 2006). Finally, cooking – the last step in meat preparation – denatures meat proteins further, reducing their water-holding capacity.

The water-holding capacity of fresh meat products is traditionally monitored by drip loss: the percentage of moisture lost over time due to gravity (Fischer, 2007). Honikel (1998) recommends using intact muscles to measure drip loss, so that the integrity of the meat sample is not influenced by forces other than gravity. Numerous drip loss methods have been established (Font-i-Furnols et al., 2015), all following the same principle – a specific portion of a muscle (or an entire muscle) is suspended in an air-tight enclosure and stored at a stable temperature for a designated amount of time. Drip loss is economically important because low drip loss implies that wholesalers and retailers can achieve higher profits as less moisture (weight) is lost throughout the value chain (Fischer, 2007).

Drip loss can be mitigated in multiple ways. First, since genetics and pre-slaughter handling play a role in *post mortem* pH decline, developing breeds that are less prone to stress and overall just reducing stressful situations pre-slaughter would help improve pH and reduce drip loss. Secondly, a combination of steep pH decline and warm muscle temperature contributes to protein denaturation (Huff-Lonergan & Lonergan, 2005); therefore, rapid chilling of carcasses *post mortem* can assist in reducing drip loss (Taylor & Dant, 1971). Furthermore, factors such as how a muscle is cut (or not) affects moisture loss. Keeping muscles intact versus cutting them into pieces reduces losses (Honikel, 1998), and when cut, the geometry matters: a small surface area to volume ratio will preserve water-holding capacity (Fischer, 2007).

A second indicator of water-holding capacity is thawing loss. Freezing leads to a disruption of fiber structure and therefore decreases overall water-holding capacity (Leygonie, Britz, & Hoffman, 2012). Freezing and subsequent thawing also appears to inconstantly affect moisture loss based on meat moisture content, rather freezing disrupts fiber structure and muscles with higher moisture content lose proportionally more fluid upon thawing (Froning, Swanson, & Richards, 1960). Although thawing losses are rarely monitored in meat science, samples are often frozen prior to analysis; therefore, making this parameter of high importance to report. In addition, thawing losses may have implications for markets with high proportions of frozen meat products and frozen convenience products containing meat, such as pizza, frozen chicken breasts, and chicken nuggets.

One way to reduce thawing losses would be to not freeze meat at all; however, freezing is the most readily implemented preservation technique for meat. Fast freezing can reduce losses upon thawing (Ngapo, Babare, Reynolds, & Mawson, 1999). In addition, lower temperatures (-80°C compared to -20°C) also decrease moisture loss upon thawing (Mortensen, Andersen, Engelsen, & Bertram, 2006). Moreover, elapsed frozen storage time increases thawing losses (Mortensen et al., 2006; Ngapo et al., 1999). Therefore, although adjustments in freezing method can be applied, they may be of little consequence for meat products frozen for extended periods of time.

Cooking loss, the moisture lost during a specific cooking process such as frying or baking, is also often assessed as an indicator of water-holding capacity. Cooking losses are more relevant at the consumer level than the wholesaler or retailer. As in drip loss, meat pH is likely the most relevant factor affecting cooking loss (Silva, Patarata, & Martins, 1999). Cooking method, cooking temperature, and end core temperature also play a role in how much yield will be left to finally eat. For example, microwaving leads to nearly 30% more moisture loss than grilling (Domínguez, Gómez, Fonseca, & Lorenzo, 2014). In addition, cooking at lower temperatures can reduce cooking loss (Christensen, Ertbjerg, Aaslyng, & Christensen, 2011; Domínguez et al., 2014). Finally, core temperature is directly linked to cooking loss; increased center temperature increases cooking loss (Combes, Lepetit, Darche, & Lebas, 2004), as well as decreases the gap in water-holding capacities between different meat qualities (Aaslyng, Bejerholm, Ertbjerg, Bertram, & Andersen, 2003).

Instrumental tenderness

Instrumental meat tenderness is the most important meat quality parameter in terms of eating quality; it mechanically mimics biting or chewing in order to estimate eating quality tenderness. Influencing meat tenderness starts with on-farm factors, such as animal breed, feeding, and physiological development (e.g., intact vs. castrated males). Meat tenderness is also affected in the slaughterhouse by animal handling, chilling rate and temperature, as well as aging. Like other physicochemical meat quality parameters, instrumental tenderness is highly correlated to pH decline (Silva et al., 1999). At the packer and retail level, tenderness depends on muscle type and cut; the type of packaging used also plays a role. Finally, cooking conditions also influence tenderness. The ability to assess tenderness has economic advantages, given that grading schemes including tenderness would allow producers and/or wholesalers to receive appropriate payment for delivered meat quality (Thompson, 2002). Therefore, research has focused on identifying factors influencing tenderness, developing techniques to increase tenderness, and the methods to best measure and model tenderness as aligned with the organoleptic (eating) experience.

The choice of breed (Christensen et al., 2011) as well as number of days on concentrate feed (McKeith, Savell, Smith, Dutson, & Carpenter, 1985; Zinn, Gaskins, Gann, & Hedrick, 1970) influences meat collagen and lipid content, which both impact instrumental tenderness. In North American beef production and in Western pork production improved tenderness is often used to justify the castration of males. Instrumental tenderness values are slightly improved with castration (Seideman, Cross, Oltjen, & Schanbacher, 1982). However, aging is more relevant in influencing instrumental tenderness; aging is necessary to attain acceptable levels of tenderness in beef (Jeremiah & Martin, 1981). In addition, longer sarcomeres, reduced connective tissue and increased levels of protein degradation increase tenderness (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002). However, when rapid cooling is implemented to improve water-holding capacity it can lead to cold-shortening of muscle fibers, which negatively impacts tenderness (Savell, Mueller, & Baird, 2005), exemplifying how compromises between quality parameters are often required. Finally, the use of highly oxygenated modified atmosphere packaging, increases the rate of protein oxidation, so that protein cross-linking leads to decreased instrumental tenderness (Bao & Ertbjerg, 2015).

Physical, chemical and enzymatic techniques have been established to improve tenderness *post mortem*. For example, the shortening of sarcomeres is known to reduce tenderness (Koohmaraie, Doumit, & Wheeler, 1996); therefore if muscle shortening during rigor could be prevented, meat tenderness would be improved. To address this, pre-rigor tenderstretching, where a carcass is hung from the hip/pelvis versus the Achilles tendon, was developed to mitigate muscle shortening (Sørheim & Hildrum, 2002). Furthermore, marinating meat and/or injection with brines or enzymes can increase tenderness by increasing water-holding capacity or fostering protein degradation, respectively (Bekhit, Carne, Ha, & Franks, 2014). In addition, cooking parameters, such as cooking temperature and time have been shown to increase the amounts of heat soluble collagen resulting in increased tenderness values (Christensen et al., 2011). For this reason, sous vide, cooking in a water bath held at a steady temperature around 55 to 60°C, is a well-suited option for improving tenderness (Botinestean, Keenan, Kerry, & Hamill, 2016). Other high input measures have been researched to increase tenderness and include high power ultrasound waves (Jayasooriya, Bhandari, Torley, & D’Arcy, 2004), high pressure processing, shockwave processing, and pulsed non-electric field (Warner et al., 2017).

There exist several physical-mechanical methods to measure instrumental tenderness; all asses the force required to penetrate a specific cooked meat sample with a specific thickness using a texture analyser (texturometer). The most commonly employed method is Warner-Bratzler Shear Force (Holman, Fowler, & Hopkins, 2016); other methods include Razor Blade Shear Force (also known as Meullenet-Owens Razor Shear; MORS), Allo-Kramer Shear Force (Xiong, Cavitt, Meullenet, & Owens, 2006), and the proposed but seldom applied slice shear force (Shackelford, Wheeler, & Koohmaraie, 1999). These methods primarily measure the force required to cut into or through a specific thickness of meat and therefore refer to shear force, as a parameter. However, texture profile analysis (TPA) has also been applied to measure tenderness. TPA mimics the process of biting and mastication using a texture analyser and returns metrics for multiple texture attributes such as hardness, springiness, and cohesiveness (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003; Ruiz De Huidobro, Miguel, Blázquez, & Onega, 2005).

Although all shear force methods are based on the same principles and correspond well with specific sensory analysis attributes (Shackelford et al., 1999; Xiong et al., 2006), instrumental tenderness values derived between the methods cannot be directly compared (Holman et al., 2016). In fact, values between different experiments using the same method are often not comparable, as method specifications significantly influence recorded values. Penetration speed, recorded shear force resistance, orientation to muscle fiber, sample thickness and/or penetration depth, and core temperature need to be maintained for comparisons to be valid (Holman et al., 2016). Instrumental tenderness data is most readily available in terms of Warner-Bratzler Shear Force; therefore, for monitoring and comparison purposes, many studies may opt for this method. However, it is the least convenient because samples are cored and then each core is sheared. Allo-Kramer involves a larger sample with a standard thickness that is sheared simultaneously with multiple steel plates. These methods are primarily used on red meat. Razor Blade Shear Force was developed to be more convenient; an intact chicken breast is penetrated with a thin blade to a specific depth. Razor Blade Shear Force has also been successfully applied with pork samples (Baublits, Meullenet, Sawyer, Mehaffey, & Saha, 2006). All three methods have been shown to correspond to well with organoleptic tenderness values (Cavitt, Meullenet, Xiong, & Owens, 2005).

Given that shear force is destructive, non-destructive technologies are desperately needed to assess tenderness on the slaughter line. Lee, Xiong, Owens, & Meullenet (2016) propose a method that involves compression versus shearing, which correlates well with shear force values. Less intrusive still are modelling techniques to estimate tenderness. Near-infrared reflectance spectroscopy has been widely studied; however efforts using this method to model tenderness remain unsuccessful (Prieto, Roehe, Lavín, Batten, & Andrés, 2009). Other modelling efforts include neural network analyses based on fresh meat images (Li, Tan, Martz, & Heymann, 1999) and carcass measurements (Hill, Jones, Robertson, & Major, 2010), which appear to be moderately successful. Finally, current research is also focused on identifying biomarkers as proxies for meat tenderness (Ouali et al., 2013).

The case of pale, soft, exudative (PSE) meat

Physicochemical meat quality parameters are often highly connected and correlated due to the biochemical pathways converting muscle to meat. The relationships between meat colour, pH, water-holding capacity, and tenderness are exemplified best by describing a common abnormality occurring in both the pork and poultry sector: pale, soft, exudative (PSE) meat. Dark, firm, dry (DFD) meat, which is just as detrimental to meat quality, is caused by similar biochemical pathways and exists in beef, and more rarely in pork. PSE starts *post mortem* with either an accelerated rate of pH decline or a below-normal ultimate pH. The (rapidly) more acidic environment leads to increased protein denaturation and reduced water-holding capacity (exudative), in terms of drip loss and thawing losses (Aaslyng et al., 2003). The additional surface moisture of the meat alters light refraction, while the reduced pH is suspected to oxidize myoglobin and oxymyoglobin to metmyoglobin; therefore, the meat loses its pink/red colour and appears pale (Adzitey & Nurul, 2011). Finally, the protein denaturation and dispelled fluid reduces tension in the meat so that the raw product is extremely soft (Adzitey & Nurul, 2011).

Some muscles are more likely to exhibit PSE characteristics, such as *pectoralis major* (breast meat) in poultry (Lesiów & Kijowski, 2003) and *longissimus lumborum* (loin) in pork (Warner, Kauffman, & Russel, 1993). Therefore, these muscles should be used to identify PSE carcass incidents. Meat colour, specifically lightness (L*), is monitored to tentatively identify PSE meat (Brewer et al., 2001) due to the non-invasive method of measurement and its strong correlation with ultimate pH. However, normal L* values vary based on genetics and geographical region. For example, an $L^* > 50$, 53, or 57 have all been documented as the cut-off values for identifying PSE cases in broiler production (Lesiów & Kijowski, 2003). In pork, L* values ranging from 60 to 66 are also considered the threshold for classifying cases of PSE meat (Adzitey & Nurul, 2011). Due to the variations that exist in normal L* values, an intermediate and/or ultimate pH should also be assessed to definitively identify PSE cases; although even pH thresholds differ between regions (Bendall & Swatland, 1988). In poultry, a $pH < 5.8$ taken at 20 minutes *post mortem* is usually the cut-off used to identify cases of PSE meat (Lesiów & Kijowski, 2003). A $pH < 6.0$ 45 minutes *post mortem* and/or an ultimate $pH < 5.3$ is used to identify PSE pork (Adzitey & Nurul, 2011).

Genetics are the leading cause of PSE meat incidences in pork and are suspected to be precursors for PSE poultry as well. The halothane gene has been identified in pork and has been linked to elevated body temperature and increased metabolism when under stress or excitement (Scheffler & Gerrard, 2007). Elevated body temperature and increased metabolism detrimentally effect meat quality by causing a faster-than-normal pH decline and accelerated protein denaturation, ultimately resulting in decreased water-holding capacity. Unfortunately, animals homozygous for the halothane gene have higher carcass weights and lean meat yields (Scheffler & Gerrard, 2007), so these animals are often preferred by producers, who are commonly paid only based on carcass weight and lean meat yield. The Rendement Napole (RN) gene has also been identified in pork and has been linked to above-normal glycogen stores, which contribute to a below-normal ultimate pH (Scheffler & Gerrard, 2007) and compromised meat quality.

The specific genes involved in incidents of PSE in poultry production have yet to be identified; however, an increase in PSE cases has been observed parallel with intensive breeding efforts towards fast growth and large breast muscle mass, implying at a genetic cause (Barbut et al., 2008). Neither the halothane gene nor the RN gene have been successfully isolated in poultry, so a different mechanism and/or unidentified genes are likely responsible for PSE in poultry (Barbut et al., 2008). To date, Malila et al. (2013) have established that differential gene expression between PSE and normal turkey meat corresponds with downregulated pyruvate production in PSE meat. Additional research is required to identify the genetic precursors of PSE in poultry. In the meantime, *ante mortem* handling, such as fasting prior to slaughter and preventing heat stress during transport are important PSE-mitigating actions in poultry production (Lesiów & Kijowski, 2003).

No matter the reason, whether inherited metabolic regulating factors or *ante mortem* stress, the case of PSE meat in both pork and poultry illustrates how a multitude of physicochemical meat quality parameters need to be assess and their relationships understood in order to appropriately classify (compromised) meat quality.

Lipid oxidation

Unlike the previously discussed physicochemical meat quality parameters, lipid oxidation is not monitored immediately *post mortem*, but becomes most relevant when discussing product shelf-life. Lipid oxidation is highly connected to fatty acid composition and atmospheric storage conditions. In short, fatty acid composition is influenced by animal type and breed, total fat deposition, fat type, and diet (Juarez et al., 2017; Wood et al., 2003). For example, poultry meat is known for having a relatively high proportion of long chain omega-3 polyunsaturated fatty acids (PUFAs) compared to pork and beef (De Smet & Vossen, 2016; Simopoulos, 2000). Pork has a higher PUFA content than beef; yet, increasing animal fat deposition can decrease total proportion of PUFAs, especially in pork (Wood et al., 2008). In addition, subcutaneous fat and adipose fat may have broadly similar fatty acid profiles compared to intramuscular lipids; nonetheless, proportions of specific fatty acids differ among fat types and across animal species (Wood et al., 2008). Finally, nearly all differences in fatty acid profile and composition are an artefact of diet (Wood et al., 2008). In light of current health-claims surrounding PUFAs, particularly omega-3 fatty acids, research has focused on incorporating feeds high in linolenic acid (C18:3) to manipulate the fatty acid composition of meat to have a lower omega-6 to omega-3 ratio (Wood et al., 2003).

However, manipulating PUFA content in red meat products can lead to adverse effects such as undesirable aromas, possibly due to increased amounts of lipid oxidation products (Kouba, Enser, Whittington, Nute, & Wood, 2003; Wood et al., 2003). In poultry, lipid oxidation of cooked meat also linearly correlates with PUFA content in raw meat (Cortinas et al., 2005). Overall, increased amounts of PUFAs result in a higher susceptibility to lipid oxidation.

Storage environment also affects lipid oxidation. High concentrations of oxygen increase the rate of lipid oxidation, like in highly oxygenated modified atmosphere packaging (HiOx MAP), which is commonly used in European markets to improve raw product colour, (McMillin, 2008). Ironically, modified atmosphere packaging is used to increase microbial shelf-life, yet it decreases non-microbial shelf-life related to lipid oxidation when the atmosphere has high concentrations of oxygen (Delles & Xiong, 2014). Lipid oxidation products are an important component of meat flavour and odour, and in high amounts (such as found in HiOxMAP packaged products) produce off-flavours and odours (McMillin, 2008). In addition, storage temperature can either mitigate (cooler temperatures) or accelerate (warmers temperatures) lipid oxidation. Rhee, Anderson, & Sams (1996) found that lipid oxidation products in beef, pork and poultry meats were up to ten times lower in samples frozen (-20°C) for 150 days compared with those stored at 4°C for 6 days.

Antioxidants have often been recommended as a mechanism to reduce lipid oxidation in meat products. They have been investigated as a dietary supplement *ante* or as a technological step *post mortem* (Falowo, Fayemi, & Muchenje, 2014). Since synthetic antioxidants can have toxicological and carcinogenic characteristics, research has focused on plant-based compounds (Kumar, Yadav, Ahmad, & Narsaiah, 2015). Tocopherols and phenolic acids, among others, have been used to successfully reduce lipid oxidation when included in animal production as a dietary supplement or applied as a technological strategy and added to processed meat products (Falowo et al., 2014; Jiang & Xiong, 2016).

Monitoring lipid oxidation is a tricky feat. Lipid oxidation is a series of chemical reactions that breaks one or more double bond in an unsaturated fatty acid, potentially saturating it with free radicals while producing new, usually volatile, compounds. This process takes place in three stages: initiation, propagation, and termination (Fernández, Pérez-Álvarez, & Fernández-López, 1997). Hydroperoxides are the most important initial products of lipid oxidation, but they are unstable and deteriorate with additional free radicals resulting in secondary products, such as pentanal, hexanal, 4-hydro-xynonenal and malondialdehyde (MDA; Fernández et al., 1997).

Because it is not feasible to monitor all resulting chemical products, multiple methods have been developed to measure a limited number of primary or secondary oxidation products. In meat science, the most commonly applied method is the determination of thiobarbituric acid reactive substances (TBARS). By spectrophotometrically measuring chemically produced fluorescent TBARS, the oxidation of linolenic acid (the most common PUFA in meat), by its secondary product MDA, can be measured (Barriuso, Astiasarán, & Ansorena, 2013). TBARS are mainly composed of a MDA-thiobarbituric acid (TBA) complex and the MDA is used as the calibration standard, but other aldehydes, carbohydrates, amino acids, and nucleic acids are also reactive with TBA, making method sensitivity, selectivity, and repeatability poor (Barriuso et al., 2013; Sørensen & Storgaard Jørgensen, 1996). TBARS have been shown to be extremely dependent on the procedure and the operator (Sørensen & Storgaard Jørgensen, 1996), making inter-study comparisons difficult. Furthermore, the TBARS method only quantifies one secondary product of linolenic acid oxidation; other oxidative products produced from other fatty acids are not monitored (Barriuso et al., 2013). Therefore, the TBARS method is ill-equipped to determine antioxidative activity (Ghani, Barril, Bedgood, & Prenzler, 2017). Despite these drawbacks, TBARS is considered an acceptable method for determining lipid oxidation in meat products and continues to be widely applied in meat science.

Other methods to monitor lipid oxidation include: iodometry to determine the peroxide value; gas chromatographic analysis of hexanal and/or other volatile secondary products; peroxide value estimates primary peroxides formed in the initial stage of oxidation and may be the oldest method used to determine lipid oxidation (Fernández et al., 1997). Hydroperoxides can also be quantified via gas chromatography-mass spectrometry; however the method is cumbersome as it involves extraction and derivatization (Barriuso et al., 2013). A more common trend is to quantify volatile secondary products by gas chromatography, which is a rapid process and can selectively quantify multiple oxidative products simultaneously. Headspace techniques are usually applied: the static headspace technique results in low oxidation product quantities which reduces quantification sensitivity; dynamic headspace is able to recover higher quantities but requires specialized equipment and extended amounts of time per sample; headspace-solid phase microextraction (SPME) is rapid and products are easily quantifiable, making it a suitable method for quantifying lipid oxidation (Barriuso et al., 2013). Given that lipid oxidation products play a key role in meat aroma, with proper procedure standardization and volatile quantification, monitoring of lipid oxidation could be co-joined with aroma analysis (which is also frequently carried out by gas chromatography) thereby stream-lining laboratory procedures.

Eating quality and sensory analysis

At the end of the day, meat is produced to be eaten. Eating quality needs to be maintained, despite what physicochemical parameters may indicate. Therefore, in order to holistically assess meat quality, organoleptic quality - food properties identified and evaluated using sensory analysis - is an important component of meat quality. There are multiple sensory tests used to assess product quality, which can be categorized into discrimination and descriptive methods (Lawless & Heymann, 2010). Discrimination tests are well-equipped to determine whether there exists a perceptible difference between two (or more) products; descriptive tests identify sensory attributes inherent to a specific product (Lawless & Heymann, 2010). The results of descriptive tests can also be employed to determine differences amongst products by comparing attribute intensities using univariate or multivariate techniques (Lawless & Heymann, 2010).

The most commonly applied method to evaluate organoleptic quality is sensory profiling by trained assessors. However, Check-All-That-Apply (CATA) and Rate-All-That-Apply (RATA) employ a relatively large sample ($n \approx 80$) of untrained assessors to profile products and have been shown to adequately profile some products (Ares et al., 2014; Oppermann, de Graaf, Scholten, Stieger, & Piqueras-Fiszman, 2017).

Sensory profiling is used to identify sensory attributes unique to a specific product. Profiling involves a group of trained assessors who become familiar with the product being tested through multiple training sessions. During the training sessions, the assessors devise the list of attributes (called the lexicon) to be evaluated for the product. The attributes are allocated reference materials so that intensity of occurrence can objectively be measured by each assessor. After multiple training sessions, the products are blindly evaluated using the lexicon and references. Quantification of attribute intensities allows for the statistical comparison of products in order to determine if listed attributes and their intensities are unique to a specific product.

The International Organization for Standardization (ISO) has recommended guidelines (ISO 13299) for conducting sensory analysis (ISO, 2016). The guidelines include use of a sensory profile, as well as partial profiles, such as a texture (ISO 11036) or spectrum® profile. In meat, partial profiling is commonly applied to monitor texture (Risvik, 1994), yet profiles have also been developed to identify off-odours and off-flavours, such as warmed-over flavour (Byrne, Bak, Bredie, Bertelsen, & Martens, 1999), and boar taint (Meier-Dinkel, Gertheiss, Müller, Wesoly, & Mörlein, 2015; Trautmann, Meier-Dinkel, Gertheiss, & Mörlein, 2016). Although less common, complete sensory profiles have successfully described numerous types of meats and their appearance, odour, flavour, and texture attributes (Rødbotten, Kubberød, Lea, & Ueland, 2004). Although sensory profiling, and more generally sensory analysis, is widely applied in meat science, procedures are often developed independently not according to ISO standards, are poorly described, and may lack attribute definitions. There exist studies where small samples of untrained consumers or research department employees act as assessors to carry out sensory analysis (Gawaad & Brune, 1979), something that should be avoided at all costs (Lawless & Heymann, 2010). Finally, descriptive analysis with trained assessors has also been carried out using hedonic scales and/or subjective preference attributes, such as 'desirability' (Caine et al., 2003; Risvik, 1994), which could contract the aim of conducting an objective analysis. Nonetheless, when properly applied the ability of sensory analysis to inherently determine organoleptic quality positions this method as the 'gold standard' to which instrumental measurements should correspond.

Partial profiling – texture profile

Although considered a partial profile, a texture profile can be highly comprehensive when carried out according to ISO standards. The ISO 11036 standard defines 8 mechanical texture attributes (Table 1) and recommends the evaluation of necessary geometrical attributes and attributes, such as moisture and fat content, at different stages of eating: visual appearance, initial bite, masticatory phase, residual phase and swallowing (ISO, 1994).

Developing a texture profile remains dynamic compared to the spectrum® profile, for which specific terminology, references, and intensity scales are pre-defined (Lawless & Heymann, 2010). Both procedures have been employed to analyse organoleptic texture, and most often results are used to validate new or novel instrumental tenderness methods and metrics (Caine et al., 2003; Cavitt et al., 2005; Cavitt, Youm, Meullenet, Owens, & Xiong, 2004; Ruiz De Huidobro et al., 2005; Xiong et al., 2006). In addition, abbreviated texture profile-like procedures that only focus on one specific sensory attribute may be carried out to validate or correlate the specific sensory attribute with physicochemical attributes; i.e. correlating juiciness at different mastication stages with moisture loss during cooking and proximate composition parameters (Aaslyng et al., 2003).

Table 1: Mechanical textural attributes, their definitions and analysis techniques as recommended in ISO 11036 (ISO, 1994)

Attribute	Definition	Technique	Frequent adjectives
Primary parameters			
Hardness	The force required to achieve a given deformation or to penetrate the product.	Perceived while compressing the product between the molars and chew evenly	Soft, hard, firm
Cohesiveness	The degree to which a substance can be deformed before it breaks.	Place the sample between two molars, compress and evaluate level of deformation prior to rupture.	See: Fracturability, Chewiness, Gumminess
Viscosity	Corresponds to the force resisting to flow.	Place a spoon with the sample directly in the front of the mouth and evaluate the force required to slurp the sample.	Fluid, thin, viscous
Springiness	The speed and degree that a sample regains its shape after deformation.	Place sample between molar teeth and partially compress; remove the force and evaluate.	Plastic, malleable, elastic, springy
Adhesiveness	Force required to remove material that adheres to the mouth.	Place samples on tongue, press against palate and evaluate force required to remove it with the tongue.	Sticky, tacky, gooey, gluey
Secondary parameters			
Fracturability (Brittleness)	Cohesiveness and force necessary to break a product into pieces.	Place sample between molars and bite down evenly until samples breaks. Evaluate the force with which the pieces move away from the teeth.	Crumbly, crunchy, brittle, crispy, crusty,
Chewiness	Cohesiveness and length of time or the number of chews needed prior to swallowing.	Place sample in mouth and masticate at one chew per second at a force equal to that needed to chew a gum drop.	Tender, chewy, tough,
Gumminess	Cohesiveness of a tender product. It is related to the effort required to disintegrate the product to a state ready to be swallowed.	Place samples in mouth and manipulate with the tongue against the palate. Evaluate the amount of manipulation necessary before food disintegrates.	Short mealy, pasty, gummy

Complete sensory profiles of meat

When experiencing meat, there are multiple facets to evaluate. Visual appearance, odour, flavour and taste coincide with texture to influence the eating experience. Appearance is usually assessed according to colour (Rødbotten et al., 2004); however visual assessment of texture attributes, such as springiness and fibrousness have also been recorded in the literature (Siekmann et al., 2018). Meat aroma is the basis for odour and flavour, which are often associated because of the body's ability to ortho-nasally (i.e., intake scent through the nose) and retro-nasally (i.e., intake scent through the mouth) experience volatile compounds while eating; retro-nasal aroma interacts with taste to produce what is perceived as flavour (Neethling, Hoffman, & Muller, 2016). Taste is clearly defined in sensory literature as being composed of sweet, salty, bitter, sour, and umami (Lawless & Heymann, 2010), all of which have been included as relevant sensory profile attributes in meat, depending on species or meat product (Baublits et al., 2006; Dermiki et al., 2013; Horsted, Allesen-Holm, Hermansen, & Kongsted, 2012). Finally, residual flavour (aftertaste) and residual texture (afterfeel) attributes have also been identified while creating sensory profiles of meat products (Baublits et al., 2006; Cullere et al., 2018; Liu, Lyon, Windham, Lyon, & Savage, 2004).

Terminology applied to odour and flavour attributes differs greatly between meat types and products and is also largely dependent on the previous experience of the assessors creating a lexicon. One commonly applied attribute in sensory tests is the species odour/flavour, e.g. 'pigginess' odour or flavour to describe pork. However, this only applies to traditionally domesticated livestock and poultry species. When it comes to game meats, sensory attribute terminology becomes more complicated; where 'beef-flavour' may be assigned to describe springbok, and the more generic term 'game' (the flavour of which is described as 'gamy') may refer to any meat that is not derived from traditional livestock (Neethling et al., 2016).

To overcome discrepancies in terminology and comprehensively describe meat without solely referring to the animal it originated from, Rødbotten et al. (2004) tested meat from 15 different species using a 22 attribute lexicon (Table 2). Unfortunately, they also relied on the term 'gamy' to describe meat odour and flavour and did not specify the reference(s) used for each attribute. Nonetheless, the attributes and progressive sensory map conceptualized remains a strong foundation for describing and assessing organoleptic meat quality on a broad scale. Sensory analysis, overall, remains a perfunctory mishmash of meat quality research composed of a limited number of studies. A focus on improving sensory analysis techniques for meat quality as well as acutely profiling the inherent attributes are needed moving forward.

Table 2: Sensory attributes identified and assessed while creating sensory profiles for meat derived from 15 different species, according to Rødbotten et al. (2004)

Sensory attribute	Definition	Highest scoring meat mean score/10 ±standard deviation	Lowest scoring meat mean score/10 ± standard deviation
Odour intensity	Intensity of sum of all odours	Hare (7.37±0.37)	Rabbit (4.20±0.20)
Sweetness odour	Odour of sugar	Beaver (4.27±0.17)	Rabbit (2.83±0.10)
Fruity acidic odour	Fruity/fresh and sour/sweet odour	Veal (4.59±0.32)	Hare (2.79±0.21)
Metallic odour	Ferrosulphate odour	Reindeer (4.22±0.22)	Rabbit (2.63±0.19)
Liver odour	Animal liver odour	Reindeer (4.51±0.31)	Chicken (1.05±0.02)
Gamy odour	Odour of wild animal	Roe-deer (5.44±0.66)	Pork (1.04±0.02)
Whiteness	Degree of white/black in fresh colour	Chicken (7.50±0.26)	Minke whale (1.90±0.15)
Colour hue	Yellow/red to red/blue in fresh meat	Beaver (7.14±0.26)	Chicken (1.69±0.11)
Colour intensity	Clear, strong colour in fresh meat	Beaver (5.42±0.15)	Chicken (1.84±0.08)
Flavour intensity	Sum of all flavours	Hare (7.20±0.20)	Rabbit (4.52±0.34)
Sweet flavour	Flavour of sugar	Roe-deer (3.93±0.23)	Pork (2.71±0.18)
Acidic flavour	Flavour of fruity/fresh and sour/sweet	Pork (4.94±0.51)	Hare (2.83±0.38)
Metallic flavour	Flavour of ferrosulphate	Reindeer (4.63±0.34)	Veal (2.79±0.19)
Liver flavour	Flavour of animal liver	Roe deer (5.54±0.45)	Pork (1.06±0.03)
Gamy flavour	Flavour of wild animal	Roe deer (5.67±0.69)	Minke whale (1.03±0.01) & Pork (1.03±0.02)
Cloying flavour	Flavour of stale, flat, sweet-like	Hare (4.01±0.30)	Pork (1.71±0.18)
Bitter	Flavour of bitter substance, like quinine	Hare (5.11±0.31)	Veal (2.69±0.15)
Coarseness	Degree of granularity of muscle fibers	Minke whale (5.11±0.13)	Roe-deer (2.47±0.20)
Hardness	Force required to bite through sample	Goat (5.24±0.93)	Lamb (2.41±0.21)
Tenderness	Time and number of chews required to masticate the sample prior to swallowing	Lamb (7.94±0.22)	Goat (4.32±1.01)
Fatness	Fatty feeling in mouth and on gums	Goat (3.10±0.40)	Rabbit (1.73±0.12)
Juiciness	Perception of water content in the sample after 3 – 4 chews	Lamb (4.68±0.47)	Turkey (2.34±0.37)

Consumer perceptions of meat quality

The most important step in meat value chains is the consumer, as meat is ultimately produced to provide a high source of protein for human diets (Font-i-Furnols & Guerrero, 2014). Yet, 'the consumer' is an abstract construct. Every consumer differs in origin and upbringing, previous product experience, attitudes and beliefs, etc., all of which make 'consumers' a very heterogeneous group with widely varying preferences. Nonetheless, some psychological factors, such as attitudes, beliefs, and expectations, can been identified as consumer-inherent factors influencing preferences for meat quality attributes (Font-i-Furnols & Guerrero, 2014). The Total Food Quality Model outlines one paradigm of how consumers perceive meat quality prior to and after purchase in regards to search, experience, and credence quality attributes (Grunert et al., 2004). Identifying the underlying psychological characteristics behind quality attribute preferences allows meat value chains to align product differentiation and processing to meet the varied consumer segment desires (Grunert, Verbeke, Kügler, Saeed, & Scholderer, 2011).

Search attributes

Search attributes, product characteristics that can be evaluated immediately by the consumer directly prior to purchase, are often the most important cues on which consumers base expectations and determine quality during purchase (Grunert et al., 2004). In the case of meat, the appearance comprises most of the search attributes. Meat appearance is composed of search attributes such as geometrical shape, marbling, colour, amount of subcutaneous fat, and so forth. Because it is a prominent search criterion for meat-buyers, retailers pay special attention to achieve and maintain what is considered "acceptable" meat colour.

Meat colour expectations and preferences differ according to species, but also according to geographical regions. In general, a bright red colour for beef, as well as pork (Lusk, Tonsor, Schroeder, & Hayes, 2018), is generally preferred by consumers, and this is the sole reason for the use of highly oxygenated modified atmosphere packaging of fresh meats (Grebitus, Jensen, & Roosen, 2013). Nonetheless, Lusk et al. (2018) identified a consumer segment in the USA that likely prefers a paler pork colour. Their explanation is that pork is often referred to as 'the other white meat' (the first white meat being chicken); therefore, some consumers may have perceived the deviation from paler/whiter meat to a stronger red colour as a reduction in quality. Kennedy, Stewart-Knox, Mitchell, & Thurnham (2005) also investigated consumer preferences for corn- vs. grain-fed chicken products in Northern Ireland and found that, unlike in the USA, consumers in Northern Ireland responded negatively to the yellow meat colour derived from corn-feeding. Similar inter-cultural discrepancies have been reported for beef colour (Grunert, 1997). Differences in meat colour preference is just one example illustrating that no single ideal of meat quality exists, especially for search attributes.

Despite appearance attributes frequently named as important decision factors (Grunert, 1997; Kennedy et al., 2004), studies focusing on consumer perception and/or acceptance of meat appearance (and its attributes) tend to be rare. Some additional achievements outside the realm of meat colour include Droval et al. (2012) and Kuttappan et al. (2012) who both investigated the extent to which consumers perceive two meat quality conditions which detrimentally impact organoleptic quality – PSE meat and white striping. White striping is an anomaly of white striations on the surface of chicken breasts usually found in fast growing heavy birds. In both cases, the authors determined that consumers were able to identify the affected products based on appearance, and affected products had reduced acceptance compared to non-compromised products.

Overall, consumers are highly sensitive to meat appearance prior to purchase, which can drastically affect product acceptance. Colour expectations for a single product may differ within a geographical region, as well as across regions. In addition, consumers are successful at identifying compromised meat quality based on appearance alone. Therefore, in the present-day when more and more consumers source their meat from the supermarket, research should focus on how to best present differing meat products for successful purchase. Differentiation of product appearance based on consumer perception and insights should be pursued (Grunert et al., 2011; Henchion, McCarthy, Resconi, & Troy, 2014).

Experience attributes

Experience attributes are largely evaluated after purchase; they pertain to eating (organoleptic) quality and preparation experience, which can only be estimated prior to purchase based on search and credence attributes. The estimated quality corresponds with expectations. Once meat has been purchased, the real experience begins, and it can be determined whether expectations are met. Experience attributes have been highly linked to consumer satisfaction and re-purchasing behaviour (Grunert et al., 2004).

As is the case with search attributes, consumer preferences for experience attributes differ throughout time and across geographic consumer segments. For example, consumer demand for meat products has shifted in the last few decades from primarily red meat products to mild, low-fat chicken breast (Henchion et al., 2014). Nowadays, chicken breast is considered versatile because it is easy to prepare, has a mild taste (so works well with a range of flavour profiles in various recipes) and is low in fat (Kennedy et al., 2004). It is also able to fulfill many of the preferred experience attributes in meat, such as convenient, tender and mild (Grunert et al., 2004; Kennedy et al., 2004) compared to redder meats.

However, this trend should not be over-generalized. Even within a species and a country, organoleptic quality preferences may differ for one type of meat. Aaslyng et al. (2007) report differences between consumers from two Danish towns. Consumers in one town prioritized tenderness and no off-flavours as important sensory attributes, while the residents of the other town emphasised fried flavour. As preferences for experience attributes are largely bound to expectations, previous experience and upbringing could largely be the basis for the varying preferences for experience attributes. Although are likely not the only factors. When Canadian consumers evaluated pork chops destined for the Japanese export market, they preferred these products in terms of overall preference, juiciness, and flavour compared to the usual domestic market product (Ngapo, Riendeau, Laberge, & Fortin, 2012). This implies that Canadian and Japanese consumers – who likely have wildly divergent past experiences and upbringings - desire similar products and that product differentiation in Canadian pork for export and domestic markets is not specifically necessary, based on consumer preferences.

As is described above, experience attributes are largely summed up by organoleptic quality, which is usually derivative of mouthfeel and flavour (Font-i-Furnols & Guerrero, 2014). Yet, preferred attributes and intensities differ drastically between groups of consumers. Therefore, in the realm of meat science and consumer research, multiple gaps remain in identifying consumer preferences for experience attributes. Identifying diverging consumer preferences will help producers differentiate their products for consumer segments, between and within countries, when necessary. Furthermore, next-to-no public research has been invested in improving the meat preparation experience based on consumer insights. There remains a rather large gap between meat product quality supplied and meeting consumer expectations.

Credence attributes

Credence attributes have increased in popularity and are steadily surpassing search attributes in importance from a consumer perspective (Grunert, 2006). These attributes are unique in that they cannot be assessed by consumers, rather consumers rely on third parties to ascertain credence attributes, such as a fair-trade or GMO-free label. Although consumers may not be able to directly evaluate them, credence attributes still heavily influence product quality perception, as illustrated through the well-established label effect (Meyerding, Gentz, Altmann, & Meier-Dinkel, 2018). In other words, products labelled with a credence attribute are likely to be valued above those without a label; however the effect of the credence attribute depends on the specific attribute and product combination (Fernqvist & Ekelund, 2014).

There are numerous credence attributes that have been associated with meat products. The most commonly applied credence attributes are related to ethics and animal welfare (Fernqvist & Ekelund, 2014). There are numerous animal welfare schemes and labels within the European market, some of which are legislated and many of which are private labels (Thorslund, Sandøe, Aaslyng, & Lassen, 2016). First, organic certification includes animal welfare measures, such as access to outdoors, and lower animal densities and is one of the reasons consumers choose organic products (Stolz, Stolze, Janssen, & Hamm, 2011). However, there do exist some standalone animal welfare labels. In the Netherlands concerns for animal welfare have often shifted and diversified production systems in favour of improved animal welfare by using both private labels and legislative changes (Elzen, Geels, Leeuwis, & Van Mierlo, 2011). German retailers also inducted private labels to signal with which products improved animal welfare is associated. As of spring 2019, a nation-wide standardized animal welfare label came into effect to mitigate consumer confusion. The mandated label divides welfare conditions into 4 categories, with organic production being the highest 'premium' category. Such a level-based scheme favours consumers, considering the valuation of consumers and therefore the gleaned quality from an animal welfare labels varies based on the label specifics and consumer socio-demographics (Clark, Stewart, Panzone, Kyriazakis, & Frewer, 2017); animal welfare issues are also perceived differently cross-nationally (Thorslund et al., 2016). In addition to the all-round animal welfare labels, studies investigating consumer preferences for specific production systems, such as extensive suckler-cow production, have found that providing information on production system attributes is essential to increasing consumer preference (Risius & Hamm, 2017).

Origin is often neglected as one of the most relevant credence attributes, which is likely linked to the perceived trustworthiness of the meat sector in a specific country. Pouta, Heikkilä, Forsman-Hugg, Isoniemi, & Mäkelä (2010) found Finnish consumers to prefer domestically produced chicken. In their study, preferences for domestic origin even outstripped production system attributes related to animal welfare. Additionally, Ngapo et al. (2012) observed that Canadian consumers sensory attribute scores climb when consumers are informed that the samples were derived from the domestic market. Finally, a similar trend was observed by

Meyerding et al. (2018) while investigating German consumer preferences for breed and origin claims; domestic identified beef steaks were preferred above premium labelled products from Argentina and Ireland.

Health claims are not widely applied for meat products, despite the large amount of research invested into improving the nutritional aspects of meat, such as total fat content and fatty acid composition. Furthermore, the effect of health claims in meat marketing are rarely a topic in research. The few studies have shown little impact of health claims on perceived meat quality and preferences (Fernqvist & Ekelund, 2014). Environmental-friendliness is also rarely assessed and included as a meat credence attribute. Van Loo, Caputo, Nayga, & Verbeke (2014) investigated one environmental claim for meat products, reduced carbon footprint, in comparison to the more frequently used credence attributes of organic, free-range, and improved animal welfare. Belgian consumers highly favoured free-range as a credence attribute compared to the other available options; improved animal welfare was also identified as an important attribute. A willingness to pay more for a reduced carbon footprint was identified. However, these results go to show that currently aspects, such as health and environmental impact, are not of highest priority for consumers.

Finally, there is a budding field of research looking into meat production inputs, such as feed. This is not trivial, given that in Germany a genetically modified organism (GMO)-free ("ohne Gentechnik") label is prevalent among large retailers. In addition, feed is often included as a credence attribute for describing chicken meat quality ('grain-fed' from Lilydale®, Canada, and 'corn-fed chicken'), as well as beef production ('grass-fed' vs. 'corn-fed' vs. 'grain-fed') in North America. Although these attributes are often used in the marketing of products, there is little research as to whether consumers truly value and use them as quality cues. Profeta & Hamm (2019b) revealed that similar to product origin, German consumers prefer animal products where locally produced feed (compared to feed imports) was included in production. Yet, the primary concern of consumers was related to the imports and incorporation of GMO feed (Profeta & Hamm, 2019a). Further research is needed to ascertain if feed remains a viable credence attribute for meat products.

Discrete choice experiments

In order to quantify consumer preferences for products encompassing multiple attributes, discrete choice analysis was developed. Discrete choice analysis is founded on attribute-utility theory (Ding, Veeman, & Adamowicz, 2015). Basically, consumers choose products that convey positive returns. These returns may endow a specific function or a 'good feeling', or some other form of gratification. Simultaneously, products may include undesirable attributes as perceived by a consumer. Consequently, the assumption is that consumers only opt for products that encompass an over-riding positive utility, and in a discrete choice scenario will choose the product returning the highest utility, i.e. with numerous and/or highly valued preferable attributes, *ceteris paribus*. In this way, utility maximization of product search, experience and/or credence attributes can be assumed to explain consumer behaviour (McFadden, 1986). The ability to sum up attributes is valuable considering that meat products are comprised of numerous attributes.

Although not the traditional medium of experimentation, there exists diverse literature investigating consumer preferences for meat products using choice experiments. Lusk & Schroeder (2004) assessed consumers' willingness to pay (WTP) for beef steak quality claims, such as 'guaranteed tender' and 'certified angus beef' and determined hypothetical choice experiments to overestimate total WTP; yet, marginal WTP across products remained in line with non-hypothetical decisions. Therefore, discrete choice experiments are useful in evaluating consumer preferences to fill the knowledge gap around consumers' preferences for specific meat quality attributes.

To date, much of the meat product literature has focussed on labeling (credence) quality attributes. With their study pertaining to broiler meat, Pouta et al. (2010) concluded that labels need to be well-established and familiar in order to have a positive impact. Similarly, in a study with trout filets from aquaculture by Risius, Janssen, & Hamm (2017), German consumers were found to prefer known and established labels relating to sustainable aquaculture production. Van Loo et al. (2014) researched Belgian consumer preferences related to environmental and ethical labelling claims often related to sustainability. They concluded that Belgian consumers prefer free range claims and are willing to pay premiums for this quality; and have weaker preferences for products labelled as 'carbon footprint' reduced. Showing a preference ranking for specific credence attributes and issues.

Further research has been conducted to determine to what extent consumers can be educated or influenced by information regarding product characteristics. Risius & Hamm (2017) found that providing information on specific animal production systems, in their case 'extensive suckler cow husbandry' positively influences preferences; although it also increases preference heterogeneity. With fish products, Bronnmann & Asche (2017) and Bronnmann & Hoffmann (2018) also established that providing information on product attributes, such as labels, strengthens preferences and results in a higher probability of purchase regarding these attributes. However, regarding packaging technology for fresh meat products, Grebitus, Jensen, & Roosen (2013) found that providing information does not result in a straight-forward link to changes in consumer preferences; although it does make them more heterogeneous.

Furthermore, a model extension has been established to include psychometrics to try and account for preference heterogeneity. Bechtold & Abdulai (2014) found attitudinal data towards functional foods to be of importance when explaining consumers' preferences towards functional dairy products. Likewise, Chen, Anders, & An, (2013) incorporated the Food Technology Neophobia Scale into their model describing Canadian consumer preferences for meat packaging technologies. In addition, Profeta & Hamm (2019b) reported decreased standard deviations when interacting their organic and local scale latent variables with the attribute 'regional feed' for multiple animal products.

Last, but not least, although discrete choice experiments present themselves as a well-suited instrument to study consumer preferences for search attributes, a limited amount of research has been carried out focussing on important consumer-identified search attributes, such as meat colour. Lusk et al. (2018) investigated US consumers' preferences for pork colour, based on quality grade labels while using pictures. However, other studies have resorted to verbally describing colour effects derived from technologies (Grebitus, Jensen, & Roosen, 2013). Unlike with experience attributes, discrete choice experiments are rapid to implement for large consumer samples and therefore are ideal for investigating consumer preferences of meat quality search attributes.

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Research Papers

Paper 1: Meat Quality Derived from High Inclusion of a Micro-Alga or Insect Meal as an Alternative Protein Source in Poultry Diets: A Pilot Study

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Abstract: The effects on meat quality resulting from alternative dietary protein sources (Spirulina and Hermetia meal) in poultry diets are studied to determine the overall suitability of these ingredients considering state-of-the-art packaging practices—highly oxygenated modified atmosphere packaging (HiOx MAP). We monitored standard slaughterhouse parameters, such as live weight, carcass weight, dressed yield, and pH at 20 min and 24 h *post mortem*. In addition, we studied the effects that 3 and 7-day storage in HiOx MAP has on the overall product physicochemical and sensory properties. In addition to previously supported effects of HiOx MAP, we found that meat quality could be improved when Spirulina replaces 50% of the soy protein in broiler diets; however, this substitution results in a dark reddish-yellowish meat colour. On the other hand, the substitution with Hermetia larval meal results in a product that does not differ from the standard fed control group, with the exception that the breast filet has a more intense flavour that decreases over storage time. All-in-all Spirulina and Hermetia meal have the potential to replace soybean meal in broiler diets without deteriorating meat quality.

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Article

Meat Quality Derived from High Inclusion of a Micro-Alga or Insect Meal as an Alternative Protein Source in Poultry Diets: A Pilot Study

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Abstract: The effects on meat quality resulting from alternative dietary protein sources (Spirulina and Hermetia meal) in poultry diets are studied to determine the overall suitability of these ingredients considering state-of-the-art packaging practices—highly oxygenated modified atmosphere packaging (HiOx MAP). We monitored standard slaughterhouse parameters, such as live weight, carcass weight, dressed yield, and pH at 20 min and 24 h post mortem. In addition, we studied the effects that 3 and 7-day storage in HiOx MAP has on the overall product physico-chemical and sensory properties. In addition to previously supported effects of HiOx MAP, we found that meat quality could be improved when Spirulina replaces 50% of the soy protein in broiler diets; however, this substitution results in a dark reddish-yellowish meat colour. On the other hand, the substitution with Hermetia larval meal results in a product that does not differ from the standard fed control group, with the exception that the breast filet has a more intense flavour that decreases over storage time. All-in-all Spirulina and Hermetia meal have the potential to replace soybean meal in broiler diets without deteriorating meat quality.

Keywords: broiler; chicken; breast meat; sensory analysis; modified atmosphere packaging; Spirulina; black soldier fly; *Hermetia illucens*; *M. pectoralis superficialis*

1. Introduction

Continued population growth and increasing income levels are driving up the demand for animal-based products and, in turn, is increasing the demand for animal feed resources [1]. Soybeans are a well-studied and widely applied as a source for protein in poultry and livestock diets. However, in recent years, concerns have mounted regarding the cultivation of soybean—particularly in topics such as world market power and the sustainability of production. Therefore, research institutions and industry alike are looking into the possible alternative animal feed protein sources available. The European Union (EU) relies on soybean imports to feed domestic poultry and livestock, with approximately 40% of animal feed protein originating from soy imports [2]. In addition, not only is the EU heavily dependent on soy imports, but China dominates the world import market by consuming approximately 41% of total world soy exports [3]. Furthermore, questions persist regarding the sustainability of soybean cultivation, primarily in the southern hemisphere. Prudêncio da Silva et al. [4] find that although deforestation is decreasing in Brazil, the previously incurred land use changes have led to secondary impacts, such as climate change and increased cumulative energy demand. Therefore, as a net importer of soy, the EU is conscious of their economic vulnerability and environmental responsibility.

This pilot study, focusing on poultry meat quality, is part of a larger project aimed at determining whether alternatives to soy can be incorporated into German meat production systems without having a negative effect on the overall product quality. Alternatives are deemed necessary in order to off-set soybean imports and decrease the European protein gap. Therefore, this study investigates the effects of two proteins that could be produced outside the arable farming system, focusing on one micro-algae source and one insect protein source, as exemplars. Spirulina (*Arthrospira platensis*) was chosen to partially replace soy because of its high crude protein content of 63% in dry matter (DM) [5] and the ability to cultivate it in photobioreactors or race-way ponds [2], which could reduce or at least limit the area required for cultivation in Europe. In addition, Spirulina contains antioxidants, such as β-carotene and vitamin E [6], which could have positive effects on meat physico-chemical parameters. *Hermetia illucens* L., also known as the black soldier fly, was also chosen as a prospective protein source for poultry diets because larvae also contain high amounts of crude protein [7] and could be fattened on numerous substrates, such as manure, cereals, and agro-food sector wastes [7,8], all of which are easily accessible in Europe, but are strictly regulated by the EU commission [9]. The conversion (recycling) of manure into a high quality animal feed [8] could be a notable advantage for Hermetia meal production; however, this is unlikely to be permitted within the EU. Unfortunately, to date in the EU, the use of animal originating feedstuffs is mostly prohibited in livestock production; nonetheless, insect feed is currently allowed in pet food and as of July 2017 has been approved for fish feed. Therefore, with continued legislative changes, Hermetia meal has the potential to be incorporated into poultry diets.

Although environmental and production sustainability are critical to ensuring the world's food supply, it should not have to come at an expense to quality. Therefore, we endeavour to capture the multi-faceted concept of meat quality through physico-chemical and sensory testing in order to determine the effects of off-setting soy with Spirulina or Hermetia meal. Ultimately, meat quality is a combination of factors [10] that differ from region to region [11] and in context [12]. Physico-chemical characteristics are usually instrumentally evaluated using literature-agreed-upon methods and parameters [13]. Whereas, sensory testing is the only way to fully quantify the aroma and texture characteristics experienced, often in combination, by consumers. This more complex testing is necessary because, of course, the palatability of meat is a combination of factors that cannot (yet) be captured simultaneously by laboratory techniques. Finally, in order to evaluate whether the alternatively-fed products can be immediately integrated into current production chains in Europe, primarily Germany, meat quality is assessed with samples stored in the industry's commonly practiced packaging [14]—highly oxygenated modified atmosphere packaging (HiOx MAP). The packaging type, length of storage time, and feed source can influence the flavour associated with aging or colour stability; therefore, in this pilot study, we investigate the impacts to physico-chemcial parameters and sensory properties when 50% of dietary soy protein is replaced by either *Arthrospira platensis* (Spirulina) or defatted dried *Hermetia illucens* larval meal (*Hermetia*) under current industrial packaging practices.

2. Materials and Methods

2.1. Animals and Diets

For this study, 132 Ross 308 male birds were raised on amino acid balanced diets where 50% of the soy-based protein was substituted by either Spirulina powder (n = 48) or Hermetia partially defatted larval meal (n = 48) in starter and grower diets; in the control group (n = 36) no soybean meal was substituted. The spirulina powder was sourced from Myanmar and had a moisture content of 3.4%. It was made up of 58.8% crude protein (DM) and 4.3% lipids (DM). The Hermetia meal was produced in Germany and the composition included 5.5% moisture, 60.5% crude protein (DM), and 14.1% lipids (DM). The main ingredients of the control diet during the starter, and respective (resp.) grower, period were wheat (32.9 resp. 37.6%), corn (16.4 resp. 18.8%), and soybean meal (39 resp. 32%). The diets were with amino acids supplemented according to the ideal amino acid ratio [15] and formulated to meet the energy and nutrient requirements of fast growing meat-type chickens according to current recommendations. Lysine level of the control diet was 1.25% and was 1.05% in the starter and grower periods. Table 1 outlines the diet composition for both starter and grower periods and the analysed diet nutritional content is listed in Table 2. The diets were created in reference to the Ross 308 nutritional specifications [16]. Further details to the procurement of Spirulina and Hermetia meal can be found in Neumann et al. [17].

Table 1. Ingredient composition of experimental diets (g/kg as fed).

Ingredients/Diets	Starter Period (1–21 day(s))			Grower Period (22–34 days)		
	Hermetia	Spirulina	Control	Hermetia	Spirulina	Control
Wheat	358.3	377.9	328.8	402.6	416.8	375.8
Corn	179.2	189.0	164.4	201.3	208.4	187.9
Soybean meal	195.0	195.0	390.0	160.0	160.0	320.0
Insect meal	145.4	-	-	119.0	-	-
Algae meal		118.2			97.0	
Soybean oil	78.5	78.5	78.5	78.5	78.5	78.5
Premix *	10.0	10.0	10.0	10.0	10.0	10.0
$CaHPO_4$	12.0	12.0	11.0	8.0	10.0	10.0
$CaCO_3$	9.9	9.1	11.0	8.0	8.0	9.0
NaCl	1.7	1.7	3.0	2.0	2.0	3.0
Wheat starch	-	-	-	3.0	3.0	-
TiO_2	-	-	-			3.0
L-Lysine·HCl	3.2	4.4	1.3	2.4	3.5	0.8
DL-Methionine	4.1	3.5	2.0	3.0	2.5	2.0
L-Threonine	0.6	-	-	0.4	-	-
L-Arginine	2.2	0.7	-	1.4	0.1	-
L-Valine	-	-	-	0.5	0.2	-

* Added per kg of final diet: 2.1 g calcium, 0.8 g sodium, 5000 IU vitamin A, 1000 IU vitamin D3, 30 mg vitamin E, 2.6 mg vitamin B1, 4.8 mg vitamin B2, 3.2 mg vitamin B6, 20 µg vitamin B12, 3 mg vitamin K3, 50 mg nicotinic acid, 10 mg calcium pantothenate, 0.9 mg folic acid, 100 µg biotin, 1000 mg choline chloride, 50 mg Fe as iron-II-sulfate, monohydrate, 15 mg Cu as copper-II-sulfate, pentahydrate, 120 mg Mn as manganese-II-oxide, 70 mg Zn as zinc oxide, 1.4 mg I as calcium iodate, hexahydrate, 0.28 mg Se as sodium selenite, 0.55 mg Co as alkaline cobalt-II-carbonate, monohydrate, and 100 mg butylhydroxytoluol.

Table 2. Analysed nutrient content of experimental diets (crude nutrients g/kg dry matter (DM)).

Diets	Starter Period (1–21 day(s))			Grower Period (22–34 days)		
	Hermetia	Spirulina	Control	Hermetia	Spirulina	Control
Crude protein	259.3	241.4	249.5	230.9	207.2	220.2
Ether extract	131.1	116.6	111.6	131.4	118.4	112.8
Crude fibre	47.1	31.1	45.2	41.7	30.4	40.4
Crude ash	60.4	59.2	65.6	56.5	53.5	61.6
N-free extract	502.1	551.7	528.1	539.5	590.5	565
AME_N (MJ/kg DM) *	15.3	15.4	14.4	15.6	15.6	14.8

* N corrected apparent metabolizable energy, calculated according to WPSA [18].

The diets were fed ad libitum and animals had constant access to water. The animals were standardly kept according to article 4 of Germany's Animal Welfare Regulation [19] on wood shaving covered floor pens (6 birds per pen; stocking density 5 birds per m^2) at the Division Animal Nutrition Physiology, University of Göttingen, Germany. In total, 8 pens per Spirulina or Hermetia treatment group were housed, and 6 pens were fed the control diet. Animals were randomized amongst the pens prior to slaughter to reduce a possible housing effect later in the experimental design. At 35 days of age, the animals were slaughtered by certified personnel at the University of Göttingen poultry slaughterhouse, which is authorized according to article 4 of the European Union's (EG) NR. 853/2004 [20]. Immediately following the slaughter, the *M. pectoralis superficialis* muscle (breast filet) was removed (excluding the *M. pectoralis profundus* muscle), and was cooled to 4 °C (ca. 5 h) until further processing, with the exception that pH and lean colour were monitored prior to cooling (see below).

2.2. Animal and Sample Management

In addition to the three feed treatment groups, the animals were further allocated into groups for physico-chemical or sensory testing, as can be seen in Figure 1. The first 12 carcasses per group (24 breast filets) were assigned for sensory testing and divided into 3 storage times so that 4 animals per feed treatment and storage time could be tested. The storage times were Fresh (not HiOx MAP packaged), 3 days, and 7 days. Two chicken filets derived from the same animal were packaged in polypropylene (PP) plastic trays (227/178/40 mm in dimension) lined with a moisture absorbent pad, heat-sealed with an oriented polyethylene terephthalate (OPET)/polypropylene (PP) film (<3 cm^3/m^2 24 h bar oxygen transmission rate; <12 cm^3/m^2 24 h bar carbon dioxide transmission rate; Dieter Seegers Haus der Verpackung GmbH, Osnabrück, Germany) and stored in an 80% O_2/20% CO_2 atmosphere. The packages were stored at 4 °C without illumination for the allotted time. Prior to sensory testing, the samples were vacuum-sealed in polyamide (PA)/polyethylene (PE) bags and frozen at −20 °C until further testing.

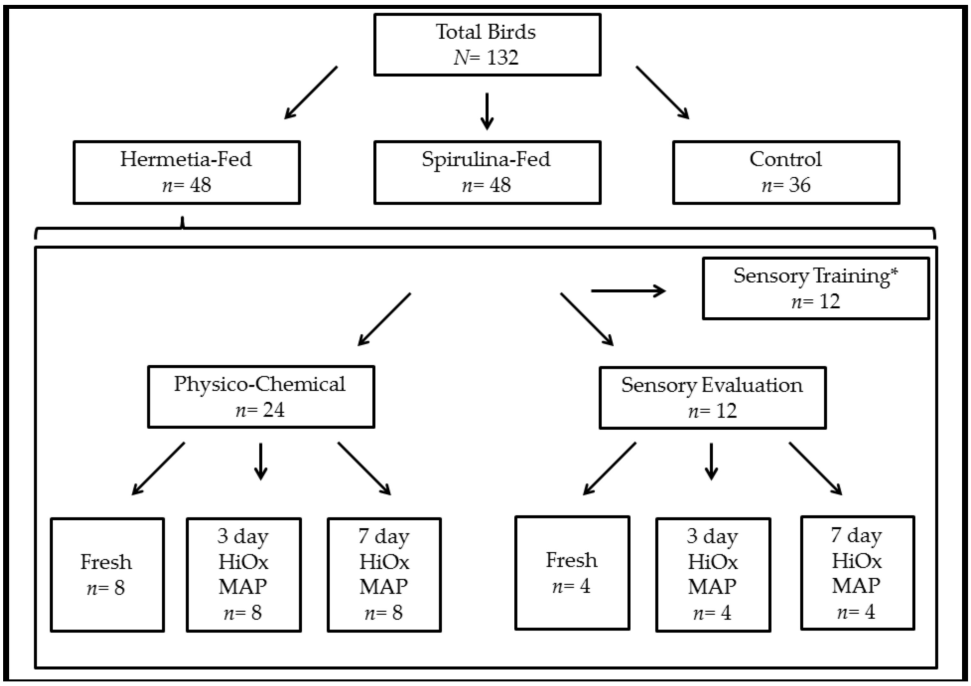

Figure 1. Allocation of birds (upper portion) and material within a treatment group (lower portion).
* Only for Spirulina- and Hermetia-fed groups

The next 24 carcasses per group were assigned for analytical meat quality testing and were also subsequently divided into 3 storage times: Fresh (n = 8), 3 days (n = 8), and 7 days (n = 8). The samples were packaged and stored as mentioned above. The following parameters were monitored: lean colour (20 min and 3 day or 7 day), pH (20 min and ultimate), lipid oxidation, storage loss (excluding Fresh samples), cooking loss, and shear force. The lipid oxidation samples were frozen at −70 °C prior to analysis and the right breast filet was frozen at −20 °C prior to cooking loss and subsequent shear force analysis. The additional animals in the Spirulina-fed (n = 12) and Hermetia-fed (n = 12) treatment groups (indicated with * in Figure 1) were also probed for pH_{20min} and pH_{24h} in the left filet, and the right filets were allocated for sensory training.

2.3. Physico-Chemical Characteristics

The pH was measured at 20 min post mortem and an ultimate pH measurement (pH_u) was taken at 24 h for the Fresh samples, and at 3 days or 7 days for the respective HiOx MAP samples. The measurements were taken with a portable pH meter equipped with a glass electrode and metal thermometer electrode (Knick Portamess 911, Berlin, Germany). The pH meter was calibrated every session prior to use using commercial pH 4 and 7 buffer solution standards (Merck, Darmstadt, Germany) at room temperature. The pH was measured by inserting the electrode completely into the superior portion of the left breast filet muscle. An accompanying thermometer was inserted alongside the electrode approximately 1 cm away. The lean colour was measured at 20 min post mortem and at 3 days or 7 days immediately after opening the package; no blooming time was allowed so that colour was recorded as close to consumers' perception through the packaging. CIELAB coordinate measurements were recorded and the values used in analysis were derived across the average of three measurements taken on the ventral side of the right breast filet using a portable spectrophotometer (model: CM 600d, Konica Minolta, Tokyo, Japan) with diffused illumination, an 8° viewing angle, and a silicon photodiode array as the detector. The spectrophotometer was calibrated using a white and black prop provided by the manufacturer prior to every session. In order to determine if the protein feed had an effect on meat composition, or whether meat composition could be significantly correlated with shear force values, breast filets were cleaned of excess subcutaneous fat, homogenized, and meat composition parameters were analysed using a Foss FoodScan™ according to Anderson [21]. To determine storage loss, the breast filets allocated for HiOx MAP were weighed prior to packaging and immediately after being removed from the package. Storage loss was expressed as the percent of weight loss over time compared to the initial sample weight. To determine cooking loss, the right breast filet, trimmed of exterior fat, was cooked sous vide for 60 min. The samples were vacuum-sealed in PA/PE bags and placed in a pre-heated hot water bath instrument (incubation/deactivation bath, Gesellschaft für Labortechnik mbH (GFL), Burgwedel, Germany) set to 77 °C. Samples were kept submerged and separated during cooking. Within the 60 min, the samples achieved 75 °C core temperatures, as determined with a preliminary study. After cooling to room temperature, the samples were weighed and cooking loss was expressed as the percentage of the initial weight. After determining cooking loss, the samples were stored at 4 °C for 24 h prior to shear force measurements. Shear force was determined according to Xiong et al. [22] with the following modifications: a TA.XTplus Texture Analyser (Stable Micro Systems, Surrey, UK) was set to a penetration depth of 15 mm to accommodate for thinner samples and a 50 N load cell was used. Each sample was tested 3 times and statistical analysis was conducted with the mean value across the 3 measurements. Shear force was measured as the highest peak (N).

2.4. Lipid Oxidation

In order to determine the extent of lipid oxidation over time, the 2-thiobarbituric acid reactive substances (TBARS) method was conducted according to Bruna et al. [23]. Results were recorded in terms of μg of malonaldehyde (MDA)/g of sample.

2.5. Sensory Analysis

Conventional profiling was used to determine the appropriate attributes to be later evaluated. Appropriate attributes were determined prior to evaluation by the assessors. In eight two-hour training sessions, the panel legitimated and defined the attributes unique amongst the products. These 19 attributes were used to evaluate the products in appearance, odour, taste and flavour, texture, and aftertaste. Overall odour intensity, animal or barn odour, metallic odour, cooked chicken odour, colour intensity (light-dark), visual elasticity assessment, fibrous appearance, overall flavour, sweet taste, sour taste, bitterness, metallic flavour, chicken flavour, overall aftertaste, hardness, juiciness, tenderness, adhesiveness, and crumbliness were evaluated (see Appendix A for more information).

The samples were cooked sous vide exactly as described for cooking loss above. The core temperature of every breast filet was checked to ensure that every sample was ca. 75 °C using a Testo 926 digital probe thermometer (Testo SE & Co. KGaA, Lenzkirch, Germany). The breast filets were then cut into 1 cm by 1cm pieces. Samples were served immediately on warmed plates and assigned with a 3 digit randomly allocated code. The evaluations took place in the University of Göttingen sensory laboratory, which is in compliance with international standard-ISO 8589 [24].

The trained panel consisted of ten assessors, who voluntarily provided written informed consent and were selected and trained according to ISO 8586-1 [25]. The panel evaluated chicken breast filet products differing in protein feed type (Spirulina, Hermetia, Control) and storage time (Fresh, 3 day HiOx MAP, 7 day HiOx MAP). In total, the nine products, derived from the three-by-three design, were evaluated in duplicate by each assessor over three one-hour sessions, where each assessor evaluated one sample for six of the nine products per session. The assessors evaluated the samples in a sequential monadic manner following four set orders that were randomly allocated, and a maximum of three assessors received the same set order during one session. The measurements were recorded electronically on 9 cm unmarked scales, a point system from 0 to 100 was stored behind the scale for later statistical analysis, using EyeQuestion survey software (Logic8 BV, Elst, The Netherlands). No assessor evaluated two samples from the same bird.

2.6. Statistical Analyses

The statistical analyses were carried out using SPSS (Version 24.0, IBM Corporation, Armonk, NY, USA) statistical software. One-way ANOVAs were conducted to determine the effect of feed on the carcass and meat quality parameters, such as live weight, carcass weight, the breast filet yield per animal, meat composition and pH_{20min} and pH_{24h}. Factorial ANOVA was carried out to determine the effects that feed, storage time (main effects), and a possible interaction effect (Feed × Storage) had on lean colour, lipid oxidation, storage loss, cooking loss, and shear force. The sensory data were evaluated using a linear mixed model with the sensory attributes as the dependent variables, feed, storage time in HiOx MAP, and a Feed × Storage interaction term as the fixed effects, and the random effects were listed as assessor and animal. Post Hoc tests were conducted using the Fischer's least significant difference (LSD) technique. Significant differences were determined where $p < 0.05$. In addition, effect size was calculated to account for possible Type 2 statistical errors, which could be likely due to the experiment's small sample size; by reporting the effect size one would nonetheless be able to compare our results to other studies and to interpret the relevance of effects as opposed to significance with the latter being substantially affected by sample size (increasing n ultimately leads to significance, even though the effect size is equally small as with a low n). Either Cohen's d for carcass performance parameters, where either Spirulina or Hermetia treatment group results were compared with the control, or partial eta-squared were calculated to estimate the effect size for physico-chemical data. Effect size was interpreted according to Cohen [26], where a small size is deemed to be 0.2 > 0.5, a moderate effect size is 0.8 < 0.5, and a large effect size is above 0.8. These values also correspond with the interpretation applied by Batorek et al. [27] in order to ascertain that the parametrical differences are derived from the treatments and not from zootechnical parameters, such as breast filet weight. Further, to maintain certainty that the significant differences were derived from the treatments, Pearson's r was also calculated between the weight of one breast filet and the corresponding dependent parameters—lean colour (L^*, a^*, b^*), lipid oxidation (TBARS), storage loss, cooking loss, and shear force, and between shear force values for Fresh samples and intramuscular fat (IMF) or moisture as dependent factors. A correlation was considered significant at a level of 0.05 using a two-tailed test.

3. Results

3.1. Physico-Chemical Results

There are only minor differences in carcass performance indicators between the feed treatment groups. Hermetia-fed birds were larger in size, which is accounted for by the statistically significant differences described in Table 3 for live weight and carcass weight. However, this difference in weight did not result in an overall increase in breast filet yield. Furthermore, the pH_{20min} and pH_{24h} values are significantly different between the different feed groups, with the control group having the highest pH values directly after slaughter, but the Spirulina-fed samples maintain a higher pH after 24 h.

Table 3. Means and standard deviation (SD) with Cohen's *d* for carcass performance parameters: live weight, carcass weight, breast filet yield, protein, intramuscular fat (IMF), moisture, and pH values at 20 min and 24 h post mortem.

Parameters	Hermetia	SD	Cohen's *d*	Spirulina	SD	Cohen's *d*	Control	SD
Live weight (g)	2329 ^{a} (n = 48)	322	0.509	2121 ^{b} (n = 48)	218	0.181	2169 ^{b} (n = 36)	306
Carcass weight (g)	1789 ^{a} (n = 48)	261	0.476	1577 ^{b} (n = 48)	175	0.465	1672 ^{b} (n = 36)	230
Breast filet yield (%)	20.4 ^{a} (n = 36)	1.9	0.205	20.0 ^{a} (n = 36)	2.0	0.400	20.8 ^{a} (n = 24)	2.0
Protein (% breast filet)	21.07 ^{a} (n = 8)	0.27	0.99	21.60 ^{a} (n = 8)	0.64	1.56	20.52 ^{a} (n = 8)	0.74
IMF (% breast filet)	3.41 ^{a} (n = 8)	0.45	0.02	3.12 ^{a} (n = 8)	0.50	0.47	3.40 ^{a} (n = 8)	0.68
Moisture (% breast filet)	73.88 ^{a} (n = 8)	0.43	0.62	73.62 ^{a} (n = 8)	0.71	0.87	74.29 ^{a} (n = 8)	0.83
pH_{20min}	6.65 ^{b} (n = 36)	0.12	0.917	6.67 ^{b} (n = 36)	0.12	0.750	6.76 ^{a} (n = 24)	0.12
pH_{24h}	5.80 ^{a} (n = 20)	0.18	0.00	6.06 ^{b} (n = 19)	0.13	1.852	5.80 ^{a} (n = 8)	0.15

Sample size indicated in brackets; superscript letter a–b indicate statistical differences between feed groups ($p < 0.05$); Cohen's *d* comparisons are between the respective treatment group and control group.

Regarding the breast filet quality, the estimated marginal means for the analysed quality characteristics are listed in Table 4. In terms of feed, nearly all differences are accounted for by the Spirulina feed. These samples were darker (L* value), redder (a* values), and more yellow (b* values) in colour compared to the Hermetia-fed and control groups. The Spirulina-fed samples lost 0.5% less moisture while being stored and were able to retain that water even after cooking, as is shown by the nearly 2% decrease in cooking loss values compared to the Hermetia-fed group and 4% decrease in values compared to the control group. The Hermetia-fed values remained similar to those of the control group across all the studied parameters. TBARS and shear force values were not significantly different across the feed groups. In addition, breast filet weight (g) correlated minimally only with the lean colour parameters L* (r = 0.589 with $p < 0.01$) and b* (r = 0.319 with $p < 0.05$).

Table 4. Estimated marginal means with standard error (SE) and effect size denoted by partial η^2 for colour, lipid oxidation (2-thiobarbituric acid reactive substances (TBARS)), storage loss, cooking loss, and shear force.

Parameters	Feed Groups			Partial η^2	Storage Time			Partial η^2	SE
	Hermetia	Spirulina	Control		Fresh	3 Day	7 Day		
L*	55.60 ^{a}	53.01 ^{b}	55.40 ^{a}	0.243	51.10 ^{f}	54.92 ^{e}	58.00 ^{d}	0.648	0.454
a*	2.00 ^{b}	3.48 ^{a}	2.43 ^{b}	0.212	0.85 ^{f}	4.04 ^{d}	3.03 ^{e}	0.554	0.261
b*	11.15 ^{b}	12.31 ^{a}	11.33 ^{b}	0.131	8.65 ^{e}	13.15 ^{d}	12.99 ^{d}	0.716	0.286
TBARS (μg/g)	0.106 ^{a}	0.095 ^{a}	0.098 ^{a}	0.006	0.081 ^{e}	0.084 ^{e}	0.134 ^{d}	0.150	0.013
Storage loss (%)	3.00 ^{a}	2.48 ^{b}	3.04 ^{a}	0.147	-	2.72 ^{d}	2.96 ^{d}	0.035	0.134
Cooking loss (%)	31.80 ^{a}	29.00 ^{b}	33.05 ^{a}	0.169	33.29 ^{d}	30.29 ^{e}	30.25 ^{e}	0.125	0.821
Shear force (N)	10.86 ^{a}	11.16 ^{a}	11.80 ^{a}	0.060	11.01 ^{d}	11.58 ^{d}	11.21 ^{d}	0.058	0.336

n = 24, except for storage loss (n = 16); superscript letter a–b indicate statistical differences between the feed groups ($p < 0.05$); superscript letter d–f indicate statistical differences between storage times ($p < 0.05$).

Concerning the effect of HiOx MAP storage, the results are not always so clear-cut. To start, the storage in HiOx MAP has an effect on the lightness values (L*) with a steady increase in the values (lightness) over the three groups. HiOx MAP also appears to increase the a* and b* values; however, these values fall slightly between day 3 and day 7. The TBARS values are not significantly different between the Fresh samples and the 3 day HiOx MAP samples; however, the values do significantly increase for 7 day HiOx MAP samples. The HiOx MAP samples were significantly different from the Fresh samples in terms of cooking loss. The HiOx MAP samples lost 3% less moisture during cooking than their Fresh counterparts. Finally, the storage losses where similar between the HiOx MAP groups, despite the four day difference in storage times, and the shear force values were similar across the groups and not significantly correlated to meat composition parameters—IMF and moisture content.

Interestingly, the only significant interaction effect (Feed × Storage) is for pH_u ($p = 0.002$). Here we can see that after the initial fall of the pH_{20min} values to the pH_{24h} values (see Table 1), the values climb back up again over time in HiOx MAP (illustrated in Figure 2). The values are the highest for the control group compared to the two treatment groups, yet remain relatively stable between the 3 and 7 day tests for all HiOx MAP samples.

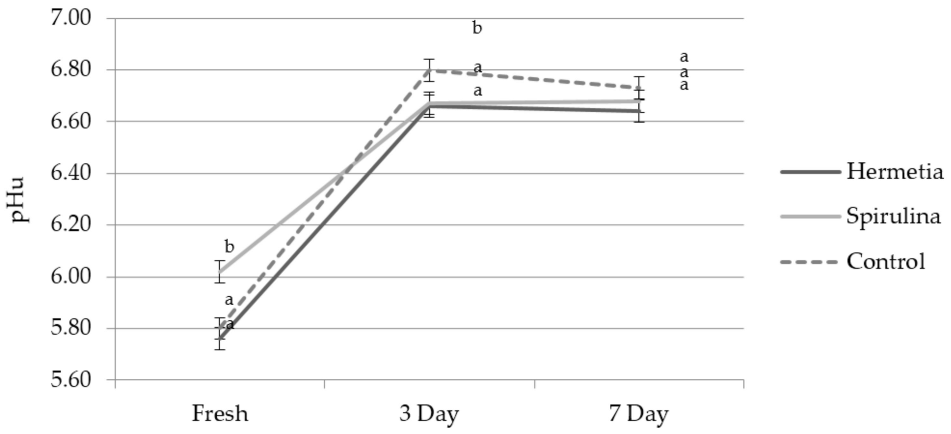

Figure 2. Interaction effect of feed and storage times ($p = 0.002$) on pH_u (mean, SE); superscript letter a–b denote statistical differences between the feed groups.

3.2. Sensory Results

Most attributes were not discernibly differentiated by the trained panel. However, the assessors determined that the feed significantly affected the hardness ($p < 0.003$) and tenderness ($p < 0.008$); storage time significantly affected elasticity when samples were stored up to 7 days ($p < 0.024$), and the interaction effect Feed × Time ($p < 0.002$) was significant for the overall flavour intensity. The effect of feed on metallic flavour was nearly significant (p-value = 0.051). Please refer to Table 5 to see the quantitative differences discerned. Generally, the Spirulina-fed samples were less metallic in flavour and the two alternative feed groups were softer and more tender than the control group.

Table 5. Estimated means with standard error (SE) and effect size (partial η^2) for the statistically significant sensory attributes (metallic flavour, hardness, and tenderness) according to feed type.

Sensory Attribute	Hermetia	Spirulina	Control	Partial η^2	SE
Metallic flavour (not metallic to strongly metallic)	20.4 ^{a}	15.8 ^{b}	17.1 ^{a}	0.223	3.90
Hardness (soft to hard)	28.8 ^{b}	25.0 ^{b}	40.8 ^{a}	0.300	4.93
Tenderness (tender to tough)	30.8 ^{b}	24.4 ^{b}	47.1 ^{a}	0.371	6.05

The table values are based on the 9 cm scale which was quantified from 0 to 100; superscript letter a–b denote statistical differences between the feed groups.

The interaction between feed type and HiOx MAP storage times is significant, and as illustrated in Figure 3 ($p = 0.002$), storage appears to have different effects depending on the feed type. For example: HiOx MAP storage appears to decrease the flavour intensity over time for Hermetia-fed samples, but not for Spirulina-fed or control samples. However, for all three groups, the 3 day HiOx MAP samples are less intense than the Fresh samples, and for the Spirulina-fed and control samples, the intensity climbs again after four additional days of aging in HiOX MAP. Finally, 7 day HiOx MAP (estimated mean of 34.0) results in a product that is less elastic compared to the Fresh (estimated mean of 41.9) and 3 day HiOx MAP (estimated mean of 41.3) breast filets (p = 0.024; SE= 5.41).

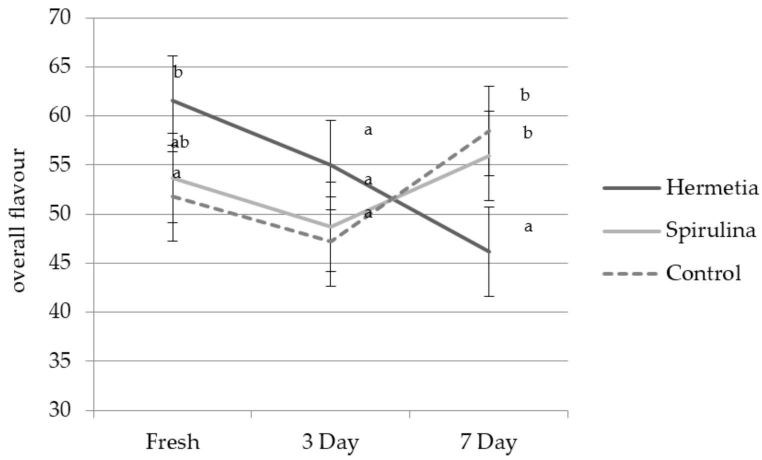

Figure 3. Interaction effect between feed and storage time on overall flavour (p = 0.002); superscript letter a–b denote statistical differences between the feed groups.

4. Discussion

The European protein gap is one reason why alternative protein sources should be widely studied [28]. Therefore, understanding the effects on meat quality from soybean meal substitution in poultry diets is important when considering what this elemental change could mean for packers, retailers, and consumers down the supply chain. At a quick glance, our results for the 50% substitution of soy-based protein with Spirulina or Hermetia larval meal, show very modest or no changes in the meat quality for many of the relevant parameters. The lack of correlation between breast filet size and the physico-chemical parameters shows that the significant differences listed above are likely not due to zootechnical differences in size between the groups, but rather due to the feed type and storage length treatments. The exceptions are L^* and b^*, where the larger filet size could influence the measureable differences; although if the filet size was the main influencing factor, then one would expect that the Hermetia-fed would be the diverging group, not the Spirulina-fed. The effect sizes, according to Cohen's d, imply that diet has a moderate effect on the carcass performance parameters, such as carcass weight and breast yield; however, the diet has a much larger effect on the pH value, especially for the Spirulina treatment group after 24 h, and type 2 statistical errors are likely not a factor with which to be concerned. According to the partial eta-squared parameters, it could be said that although feed may have a modest effect on meat quality, the type of packaging in conjunction with length of storage, in the end, plays a larger role in influencing meat quality. Although most of the results may be negligible, some of the deviations from the standard soy-fed control group should be well considered (lean colour) and appreciated (carcass weight) prior to fully incorporating alternative protein sources into poultry diets.

4.1. Spirulina in Poultry Diets

The incorporation of Spirulina results in a breast filet with a higher pH value at 24 h post mortem. In addition, storage and cooking losses decreased compared to the other two treatment groups, and this is likely related to the higher pH value. Although in our study the diets were only supplemented to balance dietary amino acids, increased dietary Lysine levels in diets has also resulted in similar findings of increased pH values and decreased storage losses [29]. Nonetheless, the result is improved meat quality, with a large effect size as according to the Cohen's d value. Reduced cooking loss should result in a breast filet that is expected to be 'juicier' [30] and more tender in texture [31]; however, sometimes these differences are first perceived by trained assessors when large differences of over 10% occur [32]. The trained panel in our study did not evaluate the Spirulina-fed breast filets as being juicier; however, Spirulina-fed breast filets are rated as being the most tender and the softest. Albeit, these traits are not significantly different as compared to those of the Hermetia-fed breast filets. Spirulina-fed breast filets also had the lowest values for metallic taste. Metallic flavour is usually considered an off-flavour in poultry products, such as white meat breast filets, given that it is a typical descriptor for game [33] and beef [34] products. Indeed, Brunton et al. [35] list high levels 1-octen-3-one as having a metallic odour associated with off-flavours in turkey breast meat, and Jayasena et al. [36] list metallic flavour as an off-flavour resulting from lipid oxidation in chicken meat. Therefore, reduced scores can be interpreted as having a positive impact on the flavour of Spirulina-fed breast filets.

The largest and most readily noticeable difference of Spirulina-fed breast filets is the significant intensive colour. Spirulina-fed breast filets are darker, redder, and more yellow in colour than the other two treatment groups tested in this study. These results are in alignment with Venkataraman et al. [37] and Toyomizu et al. [38], who noted the distinct colour of the breast filet when Spirulina was incorporated into poultry diets. The distinctive colour is not just apparent in instrumental spectrophotometer measurements, but also to the naked eye in the raw state when looking at the entire muscle. Surprisingly, when the breast filets are cooked, a colour difference between the products was not discernible by our trained panel, although we must note that it may be difficult to detect colour differences with only a 1 cm^2 surface area. The more intense colour is likely a result of the high amounts of carotenoids in Spirulina [6]. Holman and Malau-Aduli [39] believe this darker colour could be advantageous when feeding Spirulina to livestock, because meat colour is one of the most important quality indicators perceived by consumers [40–42]. However, precisely for this reason, consumer acceptance of and consumers' response to intensely pigmented poultry products needs to be studied, prior to incorporating Spirulina-fed products into the current production chain.

4.2. Hermetia in Poultry Diets

Hermetia larval meal could be used to substitute soybean meal, with minimal effects reaching the packers, retailers, or consumers. In fact, as with Spirulina, Hermetia feed could improve the overall meat quality. Hermetia animals were about 150 to 200 g heavier than their peers, and this translated into heavier dressed carcasses as well. Although the diets were calculated to be constant in caloric intake, the Hermetia meal diet was substantially higher in crude protein and ether extract (lipids), especially compared to the other treatment group, Spirulina. Therefore, it is likely that this effect is more due to the imbalances between the diets, than an inherent effect caused by Hermetia meal itself. The only other parameter that differed from the control group concerns the overall flavour intensity of Hermetia-fed breast filets. Here, a trend is noticed, where the Fresh Hermetia-fed breast filets score as the most intensive flavour; however, the intensity decreases when in HiOx MAP and over time. Although not fed with Hermetia, but rather house fly larvae, Gawaad and Brune [43] found that broiler chickens raised on (non-defatted) house fly and blow fly larvae meal had a unique smell and an intensive taste. However, this stronger taste does not need to be taken as a reduction in meat quality, because as Sheppard et al. [7] point out, some consumers prefer stronger tasting meat.

4.3. Impact of Storage Time

As previously mentioned, we packaged samples in HiOx MAP for up to 7 days, because in Germany, the country of study, this is commonly practiced by the industry for breast filet packaging and retailing [14]. We wanted to ensure that the alternatively-fed breast filets could be submitted to these conditions without reduced quality compared to the control product. In that regard, it appears that neither the Spirulina-fed nor the Hermetia-fed breast filets are negatively affected by HiOx MAP more so than the control. That being said, HiOx MAP storage over time did have some effects on overall product quality. For example, HiOx MAP increased the lightness, redness, and yellowness of the samples; although the samples were not able to maintain the complete colour change from day 3 until day 7. The values sank slightly over time. Given that the main reason for using HiOx MAP is to stimulate a more intensive red colour [42], this is not an unexpected result. Other effects include: increased TBARS values between the 3 day and 7 day HiOx MAP samples. This is expected granted that a HiOx environment induces lipid oxidation [44–48]. In addition, HiOx MAP breast filets had lower cooking losses compared to breast filets that were not packaged, but directly frozen to be analysed. This could be in part due to the additional storage time of 3 and 7 days, not specifically due to HiOx MAP. The additional aging time allowed the samples to lose about 3% of their weight; the difference in cooking loss between the HiOx MAP and Fresh breast filets is about 3%. The HiOx MAP breast filets did not lose more moisture if they were packaged for 7 compared to only 3 days. These results are similar to those of Delles and Xiong [44], who found limited changes in water-holding capacity between 4 and 14 days for HiOx MAP pork. It appears that there is a limit to storage loss amounts in HiOx MAP over time, *ceteris paribus*. Finally, 7 days of HiOx MAP impacted the elasticity of the cooked breast filets. The longer packaged breast filets were not as 'springy' when compressed with a fork and released to retake its original form. This could be due to protein oxidation [47] and a probable increase in myofibrillar deterioration [44].

4.4. Interaction Effects Feed × Storage

There are two significant interaction terms: (1) for ultimate pH and (2) for overall flavour intensity. First, the general increase in pH value in a HiOx MAP over time is supported by [43] who found that HiOx MAP increases pH values over time in pork products. However, the Spirulina- and Hermetia-fed breast filets appear to have more stable pH values in HiOx MAP compared to the control group. Although microbial indicators were not monitored in this study, according to Allen et al. [49], this could indicate that Hermetia- and Spirulina-fed breast filets could have a longer shelf-life. Secondly, the interaction term between the Hermetia-fed breast filets in HiOx MAP differs from that of the control breast filets. The Hermetia-fed breast filets taste less intense when packaged in HiOx MAP and the intensity decreases over the time that they are packaged. The Spirulina-fed and the control breast filets also decrease in flavour intensity when packaged, but the flavour intensity increases again from day 3 to day 7. This increase in intensity over time is expected granted that lipid oxidation increased between 3 and 7 days and lipid oxidation often leads to off-flavours in chicken meat [36].

4.5. Looking Forward

There are many parameters that should continue to be monitored in order to fully understand the impact that these alternative feeds have on the breast filet end product. The fatty acid composition and nutritive content, including vitamins, should be studied to determine if there are any nutritive benefits to the consumer. Studies should also monitor the pH stability of alternatively-fed products in turn with microbiological data to resolve whether these products could have longer shelf-lives than the standard fed product. In addition, focus should not be taken from determining the consumer response to products that potentially taste stronger or have a more intense colour at the point of purchase. Therefore, further research should include purchasing experiments based on poultry product colour, and more sensory testing with Hermetia-fed products should be executed. Despite the research gaps still present, we remain convinced that Hermetia and Spirulina are two good candidates to replace soybean meal in poultry diets.

5. Conclusions

Spirulina-fed breast filets generally have a higher pH, a higher water-holding capacity during storage and cooking, and have a reduced (metallic) off-flavour. The majority of the differences may be inconsequential; however, the intense colour of the Spirulina-fed breast filets should be further researched prior to incorporating Spirulina into poultry diets at a large scale. Hermetia-fed animals and carcasses are heavier and do not differ from the control group in other physico-chemical parameters. The breast filet has a more intense flavour when not HiOx packaged or aged over time, yet it remains to be seen if these differences are discernible by consumers. Based on their nominal effects on meat quality, Spirulina and Hermetia larval meal remain two potential protein alternatives for poultry diets.

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Author Contributions: Daniel Mörlein and Frank Liebert conceived the study. Carmen Neumann and Susanne Velten designed the poultry diets and contributed their time and expertise raising the birds. Brianne A. Altmann performed the meat quality physico-chemical and sensory experiments. She also analysed the data and prepared the manuscript. All authors were involved in manuscript revisions and have read and approved the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A.

Table A1. Sensory attributes identified and evaluated by trained assessors for characterizing chicken breast filets; scale from 0 to 100.

Attribute	Description	Sense	References *
1. Colour intensity	The intensity of the colour from white to dark beige	Visual	N/A
2. Elasticity	How far the sample can be pressed and still return to original form	Visual	<30: spreadable cheese 50: white bread 100: wine gum
3. Fibrousness	Fibre thickness on the product surface	Visual	<30: human hair <70: ca. 2 mm thick
4. Overall odour	The intensity of the smell when product held 2 cm from nose	Smell	N/A
5. Animal/Barn odour	Intensity of faecal, barn, or animal odour	Smell	Skatol
6. Metallic odour	Intensity of metal odour	Smell	old oxidized coins
7. Cooked chicken odour	Intensity of the odour from chicken meat in soup	Smell	cooked chicken juice
8. Overall flavour	Intensity of the overall flavour	Taste	N/A
9. Sweet taste	Intensity of sweetness	Taste	sucrose solutions 50: 6 g/L 100: 18 g/L
10. Sour taste	Intensity of sour taste	Taste	citric acid solutions 50: 0.28 g/L 100: 0.4 g/L
11. Bitter taste	Intensity of bitterness	Taste	caffeine solution 50: 0.21 g/L 100: 0.3 g/L
12. Metallic flavour	Intensity of metallic taste, such as serum or old coins	Taste	N/A
13. Chicken flavour	Intensity of taste similar to chicken soup	Taste	chicken soup broth
14. Aftertaste	The intensity of the aftertaste after swallowing	Taste	N/A
15. Hardness	The force needed to bite through the sample	Texture	<30: spreadable cheese 50: gouda cheese 100: Werther's Original
16. Juiciness	Amount of fluid released from product upon first bite	Texture	<30: raw carrot 50: cucumber 100: orange
17. Tenderness	The degree that the sample remains in one piece while chewing	Texture	<30: raw carrot 50: gouda cheese 100: hard candy
18. Adhesiveness	The degree the product sticks to your teeth; force needed to pull teeth apart	Texture	50: spreadable cheese 100: peanut butter
19. Crumbliness	Number of particles in mouth directly prior to swallowing	Texture	N/A

* Quantitative/qualitative.

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Paper 2: The Effect of Insect or Microalga Alternative Protein Feeds on Broiler Meat Quality

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Abstract: In order to combat environmental and food security concerns associated with the increasing demand for soymeal related to increasing meat consumption, this study determines the chicken meat quality derived when soymeal is substituted for either partially de-fatted *Hermetia illucens* larval meal or spirulina (*Arthrospira platensis*) in broiler diets. Physicochemical parameters, sensory traits, and fatty acid composition of the meat are investigated, as well as an experiment to evaluate the impact of highly oxygenated atmosphere versus vacuum-bag packaging on shelf life was conducted. *Hermetia illucens* did not compromise quality; meat was slightly more yellow (higher b*), had a slightly decreased pH, and was less adhesive during chewing compared to the soy-fed control. Furthermore, *Hermetia illucens* resulted in higher saturated fatty acids proportions in thigh meat. Spirulina resulted in redder (higher a*) and more yellow (higher b*) meat with a slightly increased umami and chicken flavour. Spirulina-fed chicken meat had higher lipid oxidation levels compared to the control after being packaged in a highly oxygenated atmosphere; although, differences between the spirulina-fed and control fatty acid composition in thigh meat were minor. Both alternative protein feeds show potential to replace soymeal in broiler diets; however, they do result in moderately altered products.

Research Article

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The effect of insect or microalga alternative protein feeds on broiler meat quality

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Abstract

BACKGROUND: In order to combat environmental and food security concerns associated with the increasing demand for soymeal related to increasing meat consumption, this study determines the chicken meat quality derived when soymeal is substituted for either partially de-fatted *Hermetia illucens* larval meal or spirulina (*Arthrospira platensis*) in broiler diets. Physicochemical parameters, sensory traits, and fatty acid composition of the meat are investigated, as well as an experiment to evaluate the impact of highly oxygenated atmosphere *versus* vacuum-bag packaging on shelf life was conducted.

RESULTS: *Hermetia illucens* did not compromise quality; meat was slightly more yellow (higher *b), had a slightly decreased pH, and was less adhesive during chewing compared to the soy-fed control. Furthermore, *Hermetia illucens* resulted in higher saturated fatty acids proportions in thigh meat. Spirulina resulted in redder (higher *a**) and more yellow (higher *b**) meat with a slightly increased umami and chicken flavour. Spirulina-fed chicken meat had higher lipid oxidation levels compared to the control after being packaged in a highly oxygenated atmosphere; although, differences between the spirulina-fed and control fatty acid composition in thigh meat were minor.**

CONCLUSION: Both alternative protein feeds show potential to replace soymeal in broiler diets; however, they do result in moderately altered products.

Supporting information may be found in the online version of this article.

Keywords: *Hermetia illucens*; black soldier fly; spirulina; *Arthrospira platensis*; sensory profiling; fatty acid composition

INTRODUCTION

Increasing population and incomes have driven up demand for animal-based proteins, leading to an increased need for animal feed.[1] For poultry, incorporating a high quality protein source in feed is important to ensure efficient production.[2] Conventionally, soybean meal is used as the main protein source in poultry production; however, environmental[3–6] and food security[7] concerns associated with the production of soybeans in South America have driven European research and policy to focus on alternative protein sources.[8] Two promising protein sources that could be produced in Europe include *Hermetia illucens* (HI) larvae, also known as black soldier fly, and the microalga spirulina (SP) (*Arthrospira platensis*).

HI larvae are relatively simple to reproduce and rear.[9,10] HI can be raised on various substrates, such as municipal organic[11] and vegetable[12] waste, animal waste,[13,14] and even faecal waste[15] enabling a more sustainable[16] and advantageous production compared to other insects, such as crickets which have a lower feed efficiency rate.[17] In addition, they are considered a non-pest species and therefore the environmental risk of production is minimal.[18] Finally, when considering the viability of feeding insect meal to chickens, it should be kept in mind that chicken naturally eat insect larvae[9,19] and in some regions larvae have been traditionally cultivated as a poultry protein source.[20] HI has an amino acid composition suitable for animal feed along with high levels (between 20 and 30 g kg^{-1}) of essential amino acids lysine, valine, and arginine; the larvae contain approximately 40% crude protein in dry matter.[12] However, currently in the European Union (EU) insect feed must be produced on regulated feedstuffs as a substrate, coming into direct competition with poultry production, and insect feed is only allowed in aquaculture production;[21] although permission for use in poultry production is expected in the near future. The current legal limitations mean that HI will likely remain more expensive than the current standard protein feed used in broiler production, soybean meal. Yet improvements in animal performance parameters could compensate for the additional cost of feed inputs.[22]

SP can be produced independent of arable land in bioreactors or open pond systems.[23] Currently, SP is produced to replace expensive fishmeal and fish oil supplies needed in aquaculture

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markets; however, improvements are still required to make it cost-effective as a feed ingredient at a large scale in aquaculture, and therefore in poultry production as well.[24] Bioreactors remain difficult to up-scale in production[24] and unfortunately to date SP production continues to have a larger environmental impact compared to arable crops.[25] Accordingly, much research is being invested into improving production efficiency. Examples are the integration of biogas effluent[26] or swine waste water[27] as nutrient sources and coupling production facilities to biogas reactors in order to utilize otherwise lost thermal energy.[23] The search to improve SP production is driven by its high protein content, over 60% protein in dry matter (DM),[28–30] as well as it being a source of essential fatty acids, such as linoleic acid, γ-linolenic acid, and arachidonic acid.[28,31]

The two protein sources were evaluated from a meat production perspective, monitoring parameters relevant from an industry and consumer perspective. This study investigates the effect of the inclusion of either a partially defatted HI larval meal or SP in the diets for broiler chickens on: (i) carcass traits, meat physicochemical and sensory quality; (ii) the shelf-life of meat under different packaging conditions.

MATERIALS AND METHODS

Birds and diets

Ross-308 broiler chicks were divided amongst three experimental groups; the broilers were raised on amino acid supplemented diets where 75% (starter diets) and 50% (grower diets) of the soymeal was substituted by either partially defatted HI larval meal or SP, or where no soymeal was substituted (C). One-day old broiler chicks were housed until slaughter (35 days of age) at the Division Animal Nutrition Physiology, University of Göttingen, Germany, on wood shavings in seven floor pens (seven chicks per pen at study-start; stocking density 5.8 birds m^{-2}) per treatment group. Birds were fed *ad libitum* with continuous access to water.[32] Animals were held in accordance with article 4 of Germany's Animal Welfare Regulation[33] and the study was approved (#33.9-42502-04-15/2027) by the Ethics Committee of the Lower Saxony Federal Office for Consumer Protection and Food Safety (LAVES), Germany.

The defatted HI meal was produced in Germany; whereas the SP was sourced from Myanmar. The HI meal contained 55 g kg^{-1} moisture, 141 g kg^{-1} lipids, and 605 g kg^{-1} crude protein in DM.[32] A conversion factor of 6.25 was applied to calculate crude protein content from nitrogen content. Since the time of this experiment, more accurate conversion factors have been validated for HI. A more appropriate conversion factor would be 4.76 and should be applied in future studies.[34] The SP composition included 34 g kg^{-1} moisture, 43 g kg^{-1} lipids, and 588 g kg^{-1} crude protein in DM.[32] The diets were aimed to be balanced according to the ideal amino acid ratio[35] and overall metabolizable energy.[36] This study is an extension of already published research and the diet composition in Table 1 was previously published as experiment 2 in Neumann *et al.*[32]

Dietary phosphorus (P) and calcium (Ca) contents were analysed according to standardized procedures for the chemical analysis of feedstuffs.[37] Fatty acid composition of diets was determined with freeze-dried milled (1 mm) feed samples using the procedure by Palmquist and Jenkins[38] with some modifications. In brief, 3 mL of 3 mol L^{-1} methanolic hydrochloric acid (HCl) was added to 0.5 g of feed and the mixture was incubated for 120 min at 60 °C in a water bath prior to being centrifuged at 4000 × *g* and 10 °C for 5 min. Next, 1 mL of the supernatant was removed to a test tube and 1 mL of hexane was added. The sample was gently mixed and 0.2 mL of the upper phase was used for the gas chromatography flame ionization detector (GC-FID) analysis of fatty acid methyl esters (FAME). The gas chromatograph (TRACE™ 1310) was equipped with an autosampler (AS1310) and FID; equipment was sourced from Thermo Fischer Scientific (Waltham, MA, USA). A Supelcowax™-10 (30 m × 0.32 mm × 0.25 µm; Sigma-Aldrich Chemie GmbH, Munich, Germany) capillary column was employed for the separation. Oven temperature was held at 160 °C for 1 min, increased until 220 °C (heating rate: 10 °C min^{-1}), maintained at 220 °C for 3 min, increased again until 250 °C (heating rate: 10 °C min^{-1}), and as a last step was held at 250 °C for 3 min. Each sample was injected adopting a 1:50 split ratio, at 250 °C, using hydrogen as the carrier gas with a flow rate of 1.2 mL min^{-1}. The FID operated at 260 °C with an air flow of 350 mL min^{-1}, hydrogen flow of 35 mL min^{-1}, and makeup gas flow of 40 mL min^{-1}. Fatty acids were identified using the Supelco® 37 Component FAME Mix (Sigma-Aldrich, Munich, Germany) and relative areas were analysed using the Chromeleon Chromatography Data System (Version 7.2 SR9; Thermo Fischer Scientific). All the analyses were performed in duplicate.

Sample collection

All surviving broilers were pooled according to treatment group (HI $n = 45$; SP $n = 43$; C $n = 44$) and slaughtered at 35 days of age at the University of Göttingen poultry slaughterhouse, which is regulated by article 4 of the EU's (EG) NR. 853/2004.[39] The birds were weighed, electrically stunned, slaughtered by decapitation, scalded (60 °C), defeathered, and gutted. Immediately following slaughter, the carcasses were weighed and dissected, where the breasts (*Pectoralis major*) and legs (thigh including drumstick) were skinned and cooled to 4 °C (5 h) until further analysis. The material was divided per treatment group as follows: the first 28 broilers' breasts were assigned for physicochemical analysis and the remaining eight broilers' breasts were assigned for sensory evaluation. Additional carcasses (HI $n = 9$; SP $n = 7$; C $n = 8$) were allocated for sensory panel training. Samples were stored at −18 °C for 2 months prior to sensory analysis.

The effect of highly oxygenated modified atmosphere packaging (HiOxMAP) compared to vacuum-sealed polyamide/polyethylene bags (VAC) was tested by equalling subdividing 36 birds' legs per treatment group amongst three packaging times (3, 7, and 14 days) where the right leg was VAC and the left leg was HiOxMAP packaged. The HiOxMAP packaging consisted of polypropylene (PP) plastic trays lined with a moisture absorbent pad and heat-sealed using oriented polyethylene terephthalate (OPET)/PP film (< 3 cm^3 m^{-2}/24 h/bar oxygen (O_2) transmission rate; < 12 cm^3 m^{-2}/24 h/bar carbon dioxide (CO_2) transmission rate), trapping a 80% O_2/20% CO_2 modified atmosphere. All leg samples were stored without illumination at 4 °C. The experimental unit used in analysis of meat quality was the individual bird.

Physicochemical analysis

Meat pH was recorded at 20 min and at 24 h *post mortem* using a portable pH meter (Knick Portamess 911, Berlin, Germany) equipped with a glass electrode and metal thermometer probe inserted 1 cm into the superior portion of the left breast muscle. Meat composition, including protein, moisture, and fat content were estimated using the left chicken breast trimmed of extra fat using a FOSS FoodScan™ analyser according to Anderson.[40]

Table 1. Starter and grower diet composition and analysed nutrient content as previously reported by Neumann *et al.*, experiment 2[32]

Diets	Starter diets 75% replacement			Grower diets 50% replacement		
	C	HI	SP	C	HI	SP
Ingredients (g kg^{-1} as-fed)						
Wheat	326.7	390.3	392.5	360.2	396.5	398.8
Corn	163.4	195.1	196.2	180.1	198.3	199.4
Soymeal	390	97.5	97.5	330.0	165.0	165.0
Hermetia meal	—	217.1	—	—	122.5	—
Spirulina meal	—	—	221.0	—	—	124.7
Soybean oil	78.5	58	52	91.0	80.0	76.0
Premix[a]	10.0	10.0	10.0	10.0	10.0	10.0
DCP 40	11.0	8.0	11.0	10.0	8.0	9.0
$CaCO_3$	11.0	11.0	9.0	8.0	8.0	7.0
NaCl	3.0	1.0	0.8	2.5	1.5	1.0
Wheat starch	—	—	—	3.0	3.0	3.0
L-Lysine HCl	2.5	4.2	5.8	1.8	2.8	3.6
DL-Methionine	3.6	4.2	3.5	2.6	2.9	2.5
L-Threonine	0.3	0.1	—	0.1	0.03	—
L-Arginine	—	3.5	0.2	—	1.5	—
L-Histidine	—	—	0.6	—	—	—
L-Valine	—	—	—	0.7	—	—
Analysed crude nutrients (g kg^{-1} DM)						
Phosphorus (P)	6.24	6.61	6.35	5.80	6.11	6.27
Calcium (Ca)	4.90	5.39	5.07	4.57	4.71	4.85
Crude protein	247.8	268.6[c]	262.2	236.9	224.4[c]	254.9
Ether extract	102.2	111.0	85.2	117.1	120.6	114.5
AME_N (MJ kg^{-1} DM)[b]	14.4	15.3	15.3	15.0	15.5	15.5

[a] Added per kilogram of final diet: 2.1 g calcium, 0.8 g sodium, 5000 IU vitamin A, 1000 IU vitamin D3, 30 mg vitamin E, 2.6 mg vitamin B1, 4.8 mg vitamin B2, 3.2 mg vitamin B6, 20 μg vitamin B12, 3 mg vitamin K3, 50 mg nicotinic acid, 10 mg calcium pantothenate, 0.9 mg folic acid, 100 μg biotin, 1000 mg choline chloride, 50 mg Fe as iron(II) sulphate, monohydrate, 15 mg Cu as copper(II) sulphate, pentahydrate, 120 mg Mn as manganese(II) oxide, 70 mg Zn as zinc oxide, 1.4 mg I as calcium iodate, hexahydrate, 0.28 mg Se as sodium selenite, 0.55 mg Co as alkaline cobalt(II) carbonate, monohydrate and 100 mg butylhydroxytoluol.
[b] N corrected apparent metabolizable energy, calculated according to WPSA.[36]
[c] Conversion factor of 6.25 applied; crude protein is likely reduced based on a validated reduced conversion factor of 4.76 for *Hermetia illucens* larvae.[34]

Conventional drip loss was monitored at 2 °C according to Honikel[41] using the right breast muscle hung in a polyvinyl chloride (PVC) container with a lid. The muscle was removed once at 24 h to measure lean colour development with a portable spectrophotometer (model: CM 600d, Konica Minolta, Tokyo, Japan). The average across three colour measurements was used in further statistical analysis. After 72 h *post mortem* the muscles were weighed and drip loss was expressed as a percentage of the initial sample weight.

Further, the breast was used to determine cooking loss and shear force. Trimmed of exterior fat, the breast was cooked *sous vide* at 77 °C for 60 min in a hot water bath (incubation/deactivation bath, Gesellschaft für Labortechnik mbH (GFL), Burgwedel, Germany) to reach a core temperature of approximately 75 °C. Samples were cooled to room temperature outside the vacuum bag and cooking loss was expressed as the percentage of the initial weight. Afterwards, samples were stored overnight at 4 °C until shear force was measured using the razor blade method according to Xiong *et al.*[42] The method entailed the following modifications: a TA.XTplus Texture Analyser (Stable Micro Systems, Godalming, UK) was set to penetrate 15 mm and samples were cut three times. The average over the three measurements per sample was used in further statistical analysis. Shear force was recorded as the highest peak in Newtons (N).

Thigh lean colour was measured on the lateral side of the thigh prior to and immediately after opening a stored package with a portable spectrophotometer (model: CM 600d, Konica Minolta). As with the breast muscle, the average across three colour measurements was used in further statistical analysis. Legs were weighed prior to packaging and after storage; storage loss was expressed as a percentage of the initial weight.

The 2-thiobarbituric acid reactive substances (TBARS) method from Bruna *et al.*[43] was adjusted and applied to assess lipid oxidative stability of packaged thigh meat. First, 5 mL of 20% trichloroacetic acid (TCA) were pipetted to a 0.5 g sample; 250 μL of 0.19 mol L^{-1} butylated hydroxytoluene (BHT) was added. The mixture was homogenized with an Ultra-Turrax (T18 basic, IKA®-Werke GmbH & Co. KG, Staufen, Germany) and centrifuged at 3000 × *g* and 6 °C for 6 min. Then the contents were filtered and 2000 μL of filtrate was pipetted together with 2000 μL 0.02 mol L^{-1} 2-thiobarbituric acid (TBA), shaken with a vortex mixer and incubated for 30 min at 100 °C in a water bath. After cooling for 5 min on ice, the samples were remixed, rested for 5 min at room temperature and divided into three cuvettes. The absorbance was measured using a spectrophotometer (Libra

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S22, Biochrom GmbH, Berlin, Germany) at 532 nm and malonaldehyde (MDA) concentration was expressed as μg g^{-1} of meat.

Descriptive sensory profiling

Sensory evaluation was conducted in the University of Göttingen Sensory Laboratory, which fulfils requirements set out by ISO 8589[44] and was approved by the Veterinary and Consumer Protection Agency for the Municipality and City of Göttingen. A trained panel of 12 assessors, selected according to ISO 8586-1,[45] carried out descriptive sensory profiling of the three chicken breast products: SP-fed, HI-fed, soy (C)-fed. Assessors where first exposed to the products during a training period of four 2 h sessions, where a list of attributes was collectively defined by the assessors. In total, 22 attributes were defined to be evaluated according to Siekmann *et al.*;[46] the trained panel defined three additional attributes: umami taste, fattiness; malleability (Supporting Information Table S2). Sensory evaluation was conducted using a 10 cm unmarked line-scale and data were electronically recorded by EyeQuestion survey software (Logic8 BV, Elst, The Netherlands). Products were evaluated eight times across four sessions in a complete block design. The assessors evaluated samples in a sequential monadic manner using two randomly allocated set orders per day, i.e. six assessors received the same sample order. All assessors evaluated only one sample per bird. The samples were cooked *sous vide* according to the cooking loss procedure and were cut into approximately 1 cm^2 cubes. Each sample consisted of two warm cubes on a warmed plate that was assigned a randomly allotted three-digit code.

Fatty acid composition in thigh meat

Ten of the leg samples per dietary treatment group were homogenized without adipose fat for the determination of fatty acid composition in thigh meat. The samples were prepared according to Du *et al.*[47] and the fatty acid profile was determined using GC (TRACE™ 1310) equipped with an autosampler (TriPlus RSH™) and FID sourced from Thermo Fischer Scientific. The FID was heated to 260 °C with an air flow of 400 mL min^{-1}, hydrogen (H_2) flow of 40 mL min^{-1} and a makeup gas flow of 30 mL min^{-1}. A TG-WaxMS capillary column (30 m × 0.32 mm × 0.25 μm; Thermo Fischer Scientific), was injected with 1 μL of sample with an injector temperature of 260 °C, a 1:50 split, and using helium as the carrier gas at a rate of 1.2 mL min^{-1}. The oven temperature was held at 160 °C for 1 min, increased by 20 °C min^{-1} to 200 °C, maintained for 5 min, and then increased by 10 °C min^{-1} to 230 °C (maintained for 5.5 min). The column was baked out at 30 °C min^{-1} until 250 °C for 3 min after every run. Fatty acids were identified and analysed as stated in the Birds and diets section.

Statistical analyses

One-way analysis of variance (ANOVA) was conducted to compare breast parameters and thigh fatty acid relative area means. General linear regression models were used to analyse all other leg parameter means between dietary treatments and packaging types over time; here, package system was a within-subject variable, and dietary treatment and storage time, with their interaction effect, were between-subject variables. A mixed model was applied to sensory data, where dietary treatment was the fixed effect and bird and assessor were input as random variables. Variance analyses were conducted using SPSS software (Version 24.0, IBM Corporation, Armonk, NY, USA) and statistical significance was determined with $P < 0.05$. Furthermore, principal component analysis (PCA) was used to analyse fatty acid data, as often these data are likely to be inter-correlated.[48] The data were standardized and the PCA was computed using R (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria) with the FactoMineR package.[49] Animal is the experimental unit of analysis used in this study.

RESULTS

Physicochemical parameters

Overall, the dietary treatment HI affected live and carcass weight, with HI-fed broilers being the heaviest and resulting in heavier carcasses (Table 2). SP resulted in redder (higher a^*) meat than the other two dietary treatment groups. Both alternative feeds also resulted in marginally higher b^* values (more yellow),

Table 2. Chicken breast meat physicochemical parameter means ± standard deviations across dietary protein treatments: soy (control group; C)-fed, *Hermetia illucens* (HI) larval meal-fed and spirulina (SP)-fed

Parameter	C (n = 28)	HI (n = 28)	SP (n = 28)
Live weight (kg)[a]	2.28 ± 0.41[b]	2.48 ± 0.29[a]	2.26 ± 0.36[b]
Carcass weight (kg)[a]	1.73 ± 0.34[b]	1.89 ± 0.24[a]	1.70 ± 0.30[b]
$pH_{20\ min}$	6.79 ± 0.12[a]	6.65 ± 0.17[b]	6.71 ± 0.13[ab]
$pH_{24\ h}$	5.96 ± 0.15[a]	5.84 ± 0.12[b]	5.99 ± 0.10[a]
L^*	57.0 ± 2.6	57.9 ± 1.8	57.4 ± 2.7
a^*	1.79 ± 1.35[b]	1.95 ± 1.15[b]	3.81 ± 1.18[a]
b^*	13.1 ± 1.4[b]	14.5 ± 1.0[a]	15.1 ± 1.5[a]
Moisture[b] (g kg^{-1})	754.0 ± 12.9	758.0 ± 10.6	749.5 ± 10.7
Protein[b] (g kg^{-1})	215.4 ± 11.2	209.6 ± 9.4	216.3 ± 10.0
Intramuscular fat[b] (g kg^{-1})	27.0 ± 5.0	29.5 ± 4.1	27.0 ± 4.8
Drip loss (%)	1.88 ± 0.76	1.86 ± 0.54	1.86 ± 1.01
Cooking loss (%)	24.56 ± 2.76	27.27 ± 6.05	25.89 ± 2.83
Shear force (N)	10.60 ± 2.04	9.88 ± 1.62	10.22 ± 2.27

[a] C (n = 44); HI (n = 45); SP (n = 43).
[b] n = 14 per treatment group.
[c] n = 27.
Different lowercase superscript letters indicate statistical differences between feed groups ($P < 0.05$).

compared to the control. Water-holding capacity parameters, i.e. drip loss and cooking loss, as well as shear force remained unaffected by dietary treatment.

Packaging trial with chicken thigh and attached drumstick

Lightness (*L**) values were minimally different between the different packaging systems according to dietary treatment (Fig. 1(a)). However, lipid oxidation (TBARS) levels were drastically different between the dietary treatments in HiOxMAP compared to relatively similar values in VAC (Fig. 1(b)). SP-fed samples had elevated TBARS values in HiOxMAP. Redness (*a**) colour development over time was also affected by dietary treatment (Fig. 2). Yellowness (*b**) was influenced by a combination of factors (interaction effect): packaging, storage time, and dietary treatment (Table 3). Overall SP-fed samples were the reddest throughout the entire storage period, and the control and HI-fed samples behaved similarly until the control sample redness values decreased more rapidly than those of the HI-fed samples between day 7 and day 14 (Fig. 2).

In both packaging systems, *L** values increased over time; however, values increase more intensively in VAC between 3 and 7 days, and then decreased by day 14; whereas HiOxMAP samples maintained stable *L** values between 7 and 14 days of storage (Fig. 3 (a)). Redness values were considerably higher in HiOxMAP packages across the entire storage time. Although both packaging systems values climaxed at 7 days, the differences in values between storage times are more intense, i.e. steeper slopes, in HiOxMAP (Fig. 3(b)). In addition, TBARS values peaked at 7 days for HiOxMAP packages and then decreased by 14 days; whereas VAC packaging lead to a rather steady and incremental increase in TBARS over the entire period (Fig. 3(c)). Finally, storage losses increased over time for both packaging systems, where HiOxMAP packaged samples had cumulatively higher losses until day 14 (Fig. 3(d)).

Sensory profiling

Dietary treatment affected the chicken breast sensory profile (Table 4). Chicken breast produced with SP scored higher in terms of umami and chicken flavour. It also had a reduced off-odour (barn odour). In addition, chicken breast produced with HI was less adhesive during chewing.

Fatty acid in thigh meat

HI protein feed has a significant impact on the proportion of saturated fatty acids (SFAs) found in the intramuscular fat of the chicken thighs. This increased proportion is mostly due to the increased amounts of lauric acid (C12:0) and myristic acid (C14:0), which are 100 and 10 times larger, respectively, than the thigh meat of SP-fed and control broilers (Table 5). Opposite the HI samples, SP and control thigh meat is characterized by a high proportion of polyunsaturated fatty acids (PUFAs); the control group has the highest levels of PUFAs. The superior proportions of PUFAs in the SP-fed and control samples, compared to the HI group, are largely due to an increased share of linoleic acid (C18:2 n-6).

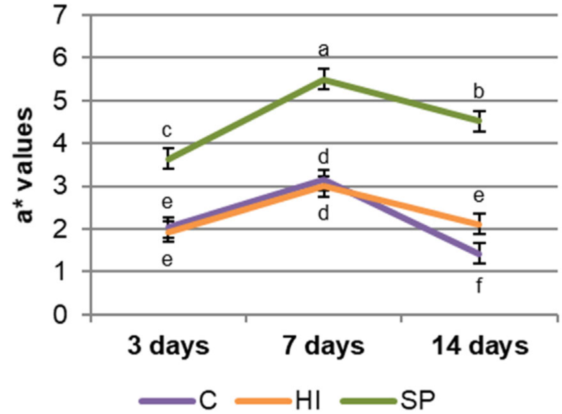

Figure 2. Estimated marginal means with standard error bars for the redness (*a**) development of leg meat samples from chickens fed *Hermetia illucens* (HI), spirulina (SP) or a soy-based control (C) feed stored up to 14 days. Different letters discern significant differences between means.

Figure 4 highlights the characterization of each treatment group based on their respective fatty acid composition. The score plot illustrates that the HI samples are unequivocally unique based on fatty acid composition; whereas there is overlap between the SP and control samples. The correlation loading plot illustrates which fatty acids correspond to which treatment group. Here it is clear that SFA content, specifically lauric acid (C12:0) and myristic acid (C14:0) are highly correlated with the HI treatment, and a high PUFA content is negatively correlated to HI feeding. Finally, the correlation loading plot depicts the unimportance played by numerous fatty acids in thigh meat intramuscular fat characterization; C20:0, C22:0, C20:3 n-6; C18:3 n-6, C22:1 n-9 are all dark in colour illustrating that their weak correlation with principal components 1 and 2.

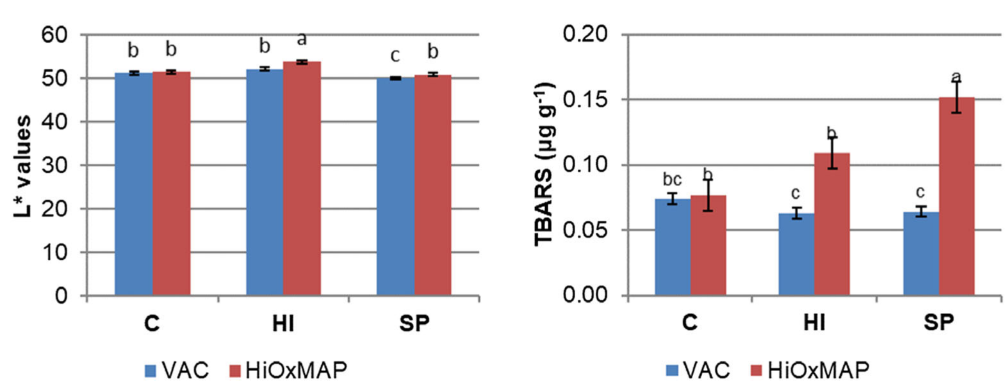

Figure 1. Estimated marginal means with standard error bars for *L** values (a) and TBARS (b) of leg meat samples from chickens fed *Hermetia illucens* (HI), spirulina (SP) or a soy-based control (C) protein source feed packaged in highly oxygenated modified atmosphere packaging (HiOxMAP) compared to vacuum-sealed bags (VAC). Different letters discern significant differences between means.

Table 3. Estimated marginal means of b^* (yellowness) values for leg pieces, originating from soy (control group; C)-fed, *Hermetia illucens* (HI) larval meal-fed, or spirulina (SP)-fed broilers, stored in vacuum-sealed bags (VAC) or highly oxygenated modified atmosphere packaging (HiOxMAP) for 3, 7, or 14 days

Package	VAC (SE = 0.38)			HiOxMAP (SE = 0.37)		
Days	C	HI	SP	C	HI	SP
3	6.87^{Bb}	7.12^{Cb}	8.33^{Ba}	10.07^{Bb}	10.46^{Cb}	11.77^{Ba}
7	8.87^{Ab}	10.56^{Aa}	10.42^{Aa}	12.32^{Ab}	11.77^{Bb}	14.64^{Aa}
14	7.87^{Ac}	9.67^{Bb}	11.15^{Aa}	10.29^{Bb}	13.14^{Aa}	13.81^{Aa}

Standard error (SE).
The VAC and HiOxMAP samples are all significantly different within each dietary treatment.
Different uppercase superscript letters indicate differences within a column (storage time).
Different lowercase superscript letters indicate significant differences within a dietary treatment for a specific packaging system (row).

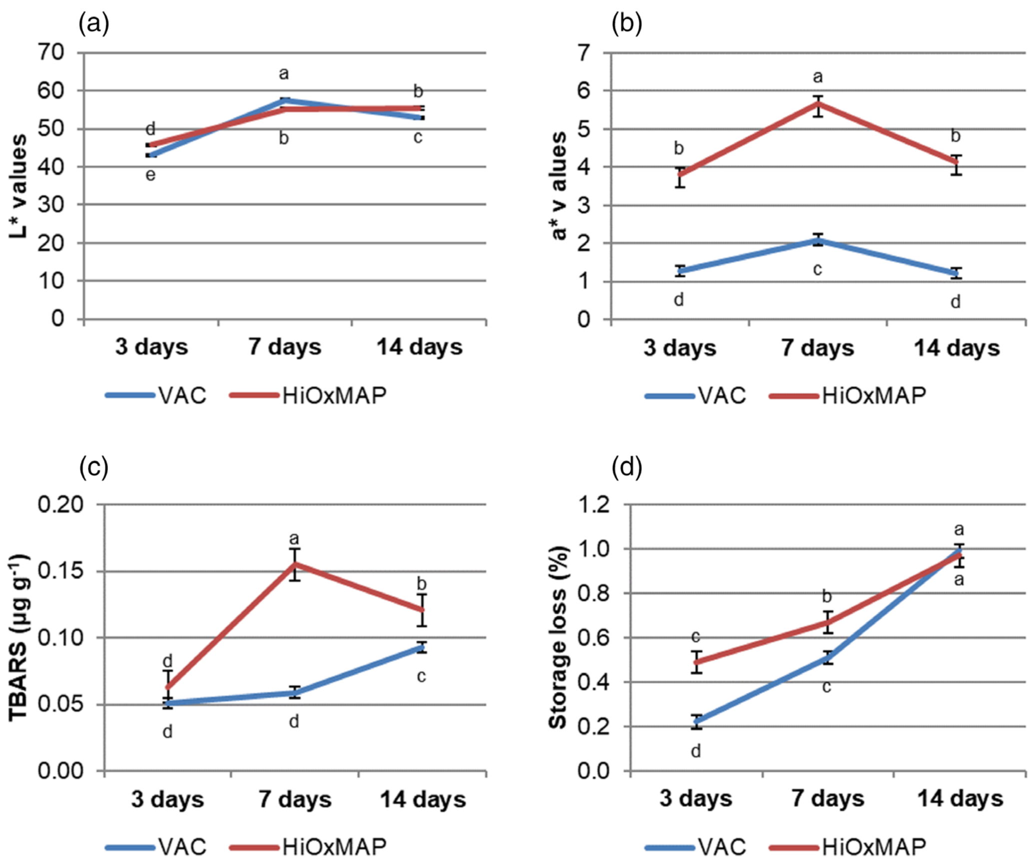

Figure 3. Estimated marginal means with standard error bars of L^* values (a), a^* values (b), TBARS (c) and storage loss (d) of leg meat in highly oxygenated modified atmosphere (HiOxMAP) or vacuum-sealed bag (VAC) packaging over 3, 7, and 14 days of storage. Different letters discern significant differences between means.

Table 4. Estimated means and standard error (SE) for sensory attributes that are significantly different between dietary protein treatments: soy (control group; C)-fed, *Hermetia illucens* (HI) larval meal-fed and spirulina (SP)-fed

Attribute	C (n = 8)	HI (n = 8)	SP (n = 8)	SE
Barn odour	14.9^{a}	15.8^{a}	11.1^{b}	2.5
Umami	18.6^{b}	18.7^{b}	21.8^{a}	3.9
Chicken flavour	56.0^{b}	55.7^{b}	59.1^{a}	5.9
Adhesiveness	47.8^{a}	43.5^{b}	48.3^{a}	6.7

Different lowercase superscript letters indicate statistical differences between feed groups ($P < 0.05$).

Table 5. Relative area (%) means ± standard deviation for identified fatty acids in thigh intramuscular fat according to dietary protein treatments: soy (control group; C)-fed, *Hermetia illucens* (HI) larval meal-fed and spirulina (SP)-fed

	C (n = 9)[a]	HI (n = 10)	SP (n = 9)
Saturated fatty acids (SFAs)	24.9 ± 0.5[b]	29.42 ± 0.7[a]	25.17 ± 1.1[b]
C10:0	ND	0.049 ± 0.007[a]	ND
C12:0	0.019 ± 0.015[b]	3.143 ± 0.420[a]	0.033 ± 0.015[b]
C14:0	0.169 ± 0.061[b]	1.223 ± 0.136[a]	0.168 ± 0.057[b]
C15:0	0.059 ± 0.006	0.059 ± 0.007	0.063 ± 0.005
C16:0	13.3 ± 0.43[b]	15.0 ± 0.67[a]	14.9 ± 0.80[a]
C17:0	0.226 ± 0.025[b]	0.154 ± 0.018[c]	0.357 ± 0.032[a]
C18:0	9.81 ± 0.86[a]	8.49 ± 0.67[b]	8.60 ± 0.72[b]
C20:0	0.037 ± 0.044	0.031 ± 0.032	0.034 ± 0.029
C22:0	0.070 ± 0.063	0.040 ± 0.062	0.062 ± 0.060
C23:0	0.331 ± 0.635	0.672 ± 0.749	0.204 ± 0.610
C24:0	1.036 ± 0.164	0.883 ± 0.162	0.862 ± 0.132
Monounsaturated fatty acids (MUFAs)	21.6 ± 0.8[b]	24.4 ± 1.0[a]	23.6 ± 1.3[a]
C14:1	0.021 ± 0.016[c]	0.190 ± 0.036[a]	0.052 ± 0.011[b]
C15:1	0.014 ± 0.022[b]	0.042 ± 0.022[a]	0.017 ± 0.026[b]
C16:1	0.447 ± 0.204	0.275 ± 0.088	0.517 ± 0.488
C17:1	0.036 ± 0.022[b]	0.035 ± 0.020[b]	0.080 ± 0.011[a]
C18:1 n-9	20.7 ± 0.9[b]	23.5 ± 1.0[a]	22.6 ± 1.2[a]
C20:1 n-9	0.113 ± 0.032	0.106 ± 0.046	0.097 ± 0.024
C22:1 n-9	0.238 ± 0.124	0.260 ± 0.148	0.291 ± 0.160
Polyunsaturated fatty acids (PUFAs)	53.5 ± 1.1[a]	46.2 ± 1.3[c]	51.2 ± 1.8[b]
C18:2 n-6	39.0 ± 0.8[a]	33.7 ± 0.8[c]	37.4 ± 1.1[b]
C18:3 n-6	2.96 ± 0.32	3.15 ± 0.72	3.24 ± 1.04
C18:3 n-3	3.43 ± 0.31[a]	2.95 ± 0.12[b]	3.06 ± 0.39[b]
C20:2 n-6	0.542 ± 0.086	0.376 ± 0.331	0.466 ± 0.049
C20:3 n-6	0.097 ± 0.093	0.117 ± 0.052	0.134 ± 0.101
C20:4 n-6	6.55 ± 0.87[a]	5.24 ± 0.83[b]	6.14 ± 0.94[ab]
C20:3 n-3	0.057 ± 0.015	0.061 ± 0.026	0.039 ± 0.023
C20:5 n-3	0.169 ± 0.033[a]	0.145 ± 0.023[ab]	0.124 ± 0.028[b]
C22:2 n-6	0.046 ± 0.137	0.056 ± 0.167	0.008 ± 0.016
C22:6 n-3	0.716 ± 0.261[a]	0.379 ± 0.113[b]	0.571 ± 0.261[ab]
Sum n-6	49.2 ± 0.8[a]	42.7 ± 1.2[c]	47.4 ± 1.7[b]
Sum n-3	4.37 ± 0.37[a]	3.53 ± 0.07[b]	3.80 ± 0.47[b]
n-6/n-3 ratio	11.3 ± 1.0	12.1 ± 0.4	12.7 ± 1.9

[a] One sample removed from analysis due to extreme deviations in fatty acid profile and composition despite running multiple aliquots.
ND, not detected.
Different lowercase superscript letters indicate statistical differences between feed groups ($P < 0.05$).

DISCUSSION

In terms of live and carcass weights, HI-fed birds were superior compared to the other two treatment groups. This finding is not supported by Pieterse *et al.*,[50] nor Onsongo *et al.*,[51] who both observed no differences in animal weights. One explanation could be imperfectly balanced energy contents between diets in this study. In a previous study, Altmann *et al.*[52] also reported increased weights for HI-fed broilers; as well as Oluokun[53] has also observed increased growth rates when feeding HI diets in compared to full-fat soybean diets. The contradictory findings could be due to unobserved differences in HI-meal composition, such as differing mineral contents or estimated crude protein contents. HI larvae contain chitin which biases standard nitrogen-to-protein conversion rates (usually 6.25 for organic matter). Janssen *et al.* have established a conversion rate of 4.76 to be more suitable for estimating crude protein content from measured nitrogen levels.[34] In the case of this study, the crude protein content of the HI grower diet is already, even with a conversion rate of 6.25, below that of the other experimental diets. Therefore, we can assume the crude protein content in diet HI to be considerably lower; nonetheless, no negative effects on neither weights nor other meat quality aspects were observed. Future studies should focus on using a conversion factor of 4.76 to verify the effects of HI on animal growth. HI larvae can also contain high amounts of P[17] and Ca,[12,54] two important minerals that need to be carefully balanced for efficient poultry development. Our HI starter diets contained slightly higher levels (increase of approximately 0.5 g kg^{-1}) of P and Ca, yet this too did not contribute to a loss in meat quality. Further research should turn its focus towards HI meal composition, expanding on knowledge regarding amino acid composition[12] and the nitrogen-to-protein conversion factor,[34] in order to best incorporate HI into poultry diets for efficient meat production.

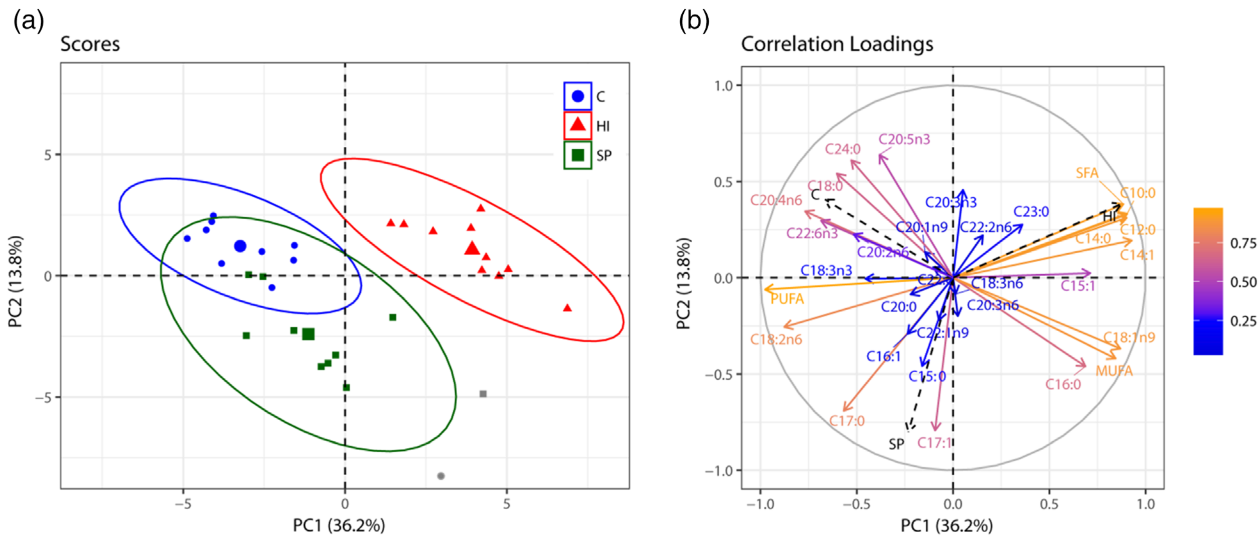

Figure 4. Score plots (a) and loading plots (b) derived from principal component analysis of fatty acid composition data from thigh meat for *Hermetia illucens* (HI), spirulina (SP) and control (C) dietary treatment groups. Variable contribution to the principal components is illustrated by arrow colour. Outliers excluded from analysis are identified in the score plot in grey.

Dietary treatment played a relatively minor role in influencing physicochemical parameters. The pH values, although significantly different between the treatment groups, remained within acceptable levels for fast-growing chicken meat.[55] In this study, chicken breast produced with HI had significantly lower pH values after 24 h *post mortem*. This is in agreement with Cullere *et al.*,[56] who reported lower ultimate pH levels for quails fed black soldier flies. Despite lower pH values, no differences between treatment groups were observed in water-holding capacity, as recorded per drip loss and cooking loss.

Lean colour is one of the noticeable physicochemical differences between the treatment groups. Venkataraman *et al.*[57] and Toyomizu *et al.*[58] have long since established that a high inclusion of SP in poultry diets significantly influences the red and yellow hues in poultry meat colour. This study confirms their results. Therefore, further research should turn its focus on the implications concerning consumer acceptance of raw chicken breast colour. In addition, chicken breasts from HI-fed broilers exhibit increased yellow (b^*) hues; this has already been observed by Altmann *et al.*[52] in raw chicken breasts. In the cooked chicken breast these differences were not discernable by a trained panel;[52] whereas Secci *et al.*[59] have determined increased b^* values for cooked Barbary partridge meat produced using HI feed.

Additionally, protein source affected lean colour values in HiOxMAP. To the best of our knowledge, Altmann *et al.*[52] remains the single study to investigate the effect of HI or SP dietary treatments throughout an industrial packaging scenario; however, that study used chicken breast instead of chicken thigh. Altmann *et al.*[52] only observed an interaction of dietary treatment with storage time for ultimate pH (not recorded in the current study), and did not observe an interaction between storage time and dietary treatment for colour parameters. The statistical differences observed in the current study remain minimal (L^* values) to moderate (TBARS) and are likely unobserved in Altmann *et al.*[52] due to the relatively small sample size. Additionally, the increased lipid oxidation levels of SP samples in HiOxMAP may be of concern, as samples could develop an oxidized flavour[60] and values observed in this study could be interpreted as above acceptable values pertaining to 'good meat quality.'[61] Unexpectedly, this increased level of lipid oxidation does not correspond with the fatty acid composition results. The fatty acid composition in intramuscular fat (IMF) of SP-fed broiler's thigh meat has a lower PUFA content than the control group. This goes to show that further research is required in determining the biochemical pathways effecting lipid oxidation in HiOxMAP packaging of poultry meat produced with SP. Protein oxidation is another important aspect that should be investigated in future studies; investigating protein quality and oxidation can also assist in this regard.[62] Moreover, it should be noted that TBARS values are method-sensitive and cannot directly be compared.[63] The other reported effects associated with HiOxMAP have already been well documented and established throughout the literature.[62,64–67]

In accordance with Pieterse *et al.*,[50] we determined no drastic differences in eating quality between the chicken breast produced with HI-fed and the control sample in eating quality when stored frozen prior to sensory evaluation. The only distinguishable difference found by our trained panel was a slightly less adhesive texture during chewing. Cullere *et al.*[68] also reported no sensory differences for broiler quail fed HI. However, some research does suggest that insect-fed meat products may have a more intensive aroma and increased juiciness attributes.[52,69]

More interestingly, higher umami and chicken flavour values were reported for SP-fed samples. Likely, these two attributes are associated. Umami is usually linked to a meaty flavour[70] and SP's profile includes a strong umami taste.[71] Increased chicken flavour and umami taste is likely favourable in chicken meat products. Limited research has investigated the consumer acceptance of the eating quality resulting from SP as a feed in poultry diets; yet, in red sea bream Mustafa *et al.*[72] indicate that taste is improved with just a 2% contribution of SP in the fish diet. However, Nandeesha *et al.*[73] report no discernable difference in flavour. More profound studies need to incorporate sensory profiling coupled with consumer sensory tests in order to better understand the effects of SP protein feed on eating quality.

Finally, HI significantly affected the fatty acid composition in chicken thigh meat. The high levels of C12:0 and C14:0 found in the feed (Table S1) are reflected in the fatty acid composition of the thigh meat. Just as in Cullere *et al*.[68] with quail broilers, Secci *et al*.[59] with Barbary partridge, and Schiavone *et al*.[74] with broiler chickens fed HI fat (not meal), C12:0 and C14:0 levels increased in our study compared to a soy-based control group. In addition, 14:1 also characterized HI-fed samples; whereby Secci *et al*.[59] found no differences in this fatty acid in the raw or cooked samples of Barbary partridge, yet Cullere *et al*.[68] and Schiavone *et al*.[74] also reported increased C14:1 levels. Surprisingly, Pieterse *et al*.[50] did not report any significant differences in the fatty acid composition of cooked chicken broiler meat produced with diets containing HI.

Finally, although SP is often marketed as a good source of PUFAs, the SP-fed chicken in our study did not produce thigh meat with the highest PUFA content. In fact, the control samples contained the highest proportions of PUFAs. Furthermore, the control treatment had the largest proportion of omega-3 fatty acids. SP is cited as high in γ-linolenic acid and other essential omega fatty acids;[28,30] however levels vary widely.[31] On the other hand, soy is known to have high levels of polyunsaturated fats, such as linoleic and linolenic acids.[75,76] In this regard soy is already a reasonable feed component to ensure a high content of good PUFAs. Therefore, the expectations of increasing PUFA content in chicken meat by feeding SP should not be over-estimated. Especially considering that the SP feed used in this study was high in omega-6 fatty acids, not omega-3 fatty acids. Other feed ingredients have been established to increase the omega-3 fatty acid content in poultry.[77] Particularly, Gatrell *et al*.[78] were able to successfully increase the PUFA content in chicken thigh, especially the omega-3 fatty acids content, using microalgal biomass derived from *Nannochloropsis oceanica*. SP has been shown to influence the PUFA[79] and overall fat content[80] in animal products; however, the next step should focus on increasing the omega-3 fatty acid proportion of the microalga so that SP could play a more relevant role in improving fatty acid composition of poultry products.

CONCLUSIONS

Our experiment clearly portrays the resulting poultry meat quality between a partially-defatted HI meal and SP-based diets, compared to a soy-fed control. Results show that the alternative protein sources can be viably included in broiler chicken production, as investigated from a multi-faceted meat quality perspective. However, both alternative protein sources come with their own challenges. HI-fed meat contains an increased proportion of SFAs, which may increase quality parameters, such as shelf-life, but are not positively perceived from a health point of view. In the case of SP, special attention should be paid to the intensive meat colour and increased lipid oxidation of products in HiOxMAP when planning to bring these products to market. All-in-all, no eating quality concerns were identified. HI-fed chicken mirrored the control group, and SP feed makes it taste all-the-more like chicken.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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Author Contributions: Prof. D. Mörlein conceived the over-arching project. Ms. B. Altmann designed carried-out the meat quality physicochemical and sensory experiments. Ms. R. Wigger conducted fatty acid composition analysis with Dr. M. Ciulu. Ms. B. Altmann analysed the data and prepared the manuscript. Prof. D. Mörlein was involved in manuscript revisions and has read and approved the manuscript.

Paper 3: Do Dietary Soy Alternatives Lead to Pork Quality Improvements or Drawbacks? A Look into Micro-Alga and Insect Protein in Swine Diets

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Abstract: Pork quality characteristics related to the dietary substitution of soybean meal by the micro-alga Spirulina (*Arthrospira platensis*) or black soldier fly (*Hermetia illucens*) partly-defatted larval meal were observed. Through a duplicated study totaling 48 individually-fed barrows (Pietrain × (Large White × Landrace)) allocated into two experimental groups and a control, the effect of dietary protein source on physicochemical and sensory pork quality was monitored under current industrial packaging conditions (highly-oxygenated modified atmosphere packaging). The results show that physicochemical characteristics are not degraded by including alternative protein sources in pig diets. *Hermetia illucens* increased lauric acid levels in backfat indicating that this fatty acid may be suitable as a biomarker for *Hermetia illucens*-fed pork. This goes to show that protein alternatives do not compromise pork quality.

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Do dietary soy alternatives lead to pork quality improvements or drawbacks? A look into micro-alga and insect protein in swine diets

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ABSTRACT

Pork quality characteristics related to the dietary substitution of soybean meal by the micro-alga Spirulina (*Arthrospira platensis*) or black soldier fly (*Hermetia illucens*) partly-defatted larval meal were observed. Through a duplicated study totalling 48 individually-fed barrows (Pietrain × (Large White × Landrace)) allocated into two experimental groups and a control, the effect of dietary protein source on physico-chemical and sensory pork quality was monitored under current industrial packaging conditions (highly-oxygenated modified atmosphere packaging). The results show that physico-chemical characteristics are not degraded by including alternative protein sources in pig diets. *Hermetia illucens* increased lauric acid levels in backfat indicating that this fatty acid may be suitable as a biomarker for *Hermetia illucens*-fed pork. This goes to show that protein alternatives do not compromise pork quality.

1. Introduction

To feed a growing population with increasing animal protein demands, a production shift needs to take place. One of the most environmentally detrimental steps in meat production is the production of feedstuffs, in particular land use change (Reckmann, Blank, Traulsen, & Krieter, 2016) and land occupation (Mungkung et al., 2013). This is especially prudent given that the area of arable land per person will continue to decrease moving forward. The limited area available for cultivation is a relevant problem throughout Europe as is exemplified through the infamous ‘protein gap’ and the European Union's (EU) dependence on soybean imports. Alternatives to the land-based production of soybean meal as the customarily utilized protein source in animal feed will be required moving forward.

Micro-algae and insect meals present themselves as two potential alternatives, as they could both be produced independent of arable land and are high in protein. In this study we focus on one micro-alga, Spirulina (*Arthrospira platensis*), and on larval insect meal derived from *Hermetia illucens* as potential animal feed protein sources. Spirulina contains a superior crude protein content of 63% in dry matter (DM) (Tokusoglu & Unal, 2003) compared to a standard soybean meal used in animal feeds (Barroso et al., 2014). Unlike soybeans, Spirulina does not require arable land, but is produced in a water culture in photobioreactors or race-way ponds (Taelman, De Meester, Van Dijk, da Silva, & Dewulf, 2015) and is thereafter dried resulting in a powder that can be immediately integrated into animal diets. In addition, Spirulina contains antioxidants, such as β-carotene and vitamin E (Habib, Parvin, Huntington, & Hasan, 2008) and is known to have a high content of gamma linolenic acid (Diraman, Koru, & Dibeklioglu, 2009), which could result in increased levels of this omega-6 fatty acid in the end product.

The nutritional components of *Hermetia illucens*, also known as the black soldier fly, have been well studied (Barroso et al., 2014; Makkar, Tran, Heuzé, & Ankers, 2014; Sánchez-Muros, Barroso, & Manzano-Agugliaro, 2014; Spranghers et al., 2017; Wood et al., 1999) also making it a potential protein source for animal feed. Larvae contain about 40% crude protein in DM (Spranghers et al., 2017), but this can be drastically increased if the larvae are defatted prior to inclusion in livestock diets. *Hermetia illucens* also presents a comparable amino acid composition compared to that of soybean meal (Barroso et al., 2014) and the larvae can be cultivated on numerous substrates, many of which are considered agricultural bi- or waste products (Newton, Sheppard, & Burtle, 2008; Spranghers et al., 2017; St-Hilaire et al., 2007). Although the incorporation of such substrates could improve the overall sustainability aspects of *Hermetia illucens* cultivation (Smetana, Palanisamy, Mathys, & Heinz, 2016), it is unlikely that at present agro-food wastes or manure would be permitted as a substrate in the EU. Especially given that currently the use of animal originating feedstuffs,

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therefore including insects, is strictly prohibited; exceptions have been made thus far for fish feed (Commission Regulation (EU), 2017/893) and pet food. Nonetheless, in the future *Hermetia illucens* could become an important soy substitute in livestock diets.

This study goes beyond the usual research on the animal nutrition parameters and focuses on product quality, as would be encountered by the processors or consumers. We examine the effect that feeding alternative protein sources may have on pork products, given its worldwide importance and its popularity as the most consumed type of meat in Germany (Schmidt-Landenberger, 2018), the country hosting this study. Physico-chemical as well as sensory parameters are monitored to determine whether the alternative protein sources in question result in compromised product quality or could be directly integrated into the current state-of-the-art production system. In Germany, current retail conditions heavily rely on pre-packaged products, where highly-oxygenated modified atmosphere packaging (HiOxMAP) is employed (BfR, 2010). These products are often packaged directly at the meat processor and obtain an expiry date of up to 14 days (processor and product dependent). Therefore, we include HiOxMAP in our research design, given that a potential consumer is likely to encounter such a product in a retail chain and an altered fatty acid composition towards more unsaturated fatty acids could affect shelf-life characteristics, such as rancidity (Wood et al., 2004). In addition, research is looking into possible mechanisms to improve the nutritional quality of red meat. One of these improvements could include a fatty acid composition high in polyunsaturated fatty acids (Wood et al., 2004). Therefore, fatty acid profiles of the alternatively-fed products are examined to rectify lipid oxidation findings and more comprehensively characterize the carcasses.

To date, only one short study has investigated pork quality parameters with the dietary inclusion of Spirulina; although fatty acid composition was not included (Simkus et al., 2013). Additionally, there have been no studies on the effect of *Hermetia illucens* in pork diets pertaining to meat quality. Our study is multifaceted, so as to comprehensively evaluate pork quality, which cannot be done alone through physico-chemical parameters. Sensory testing is a vital component of quality testing to determine how and whether differences in physico-chemical traits could be perceived by sensitive end consumers. Especially given that eating quality is one of the pre-requisites to consumer product choice (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). Therefore, this study aims to ascertain the possible improvements in or drawbacks to physico-chemical and sensory characteristics of pork resulting from alternative protein sources in pork diets, with special consideration to the current industrial retail packaging conditions.

2. Materials and methods

2.1. Animals and diets

In total, 48 (Pietrain × (Large White × Landrace)) barrows were divided amongst three diets within two experimental replicates. This equals eight animals per diet per replicate and 16 animals overall per diet. One animal mortally injured itself from the *Hermetia illucens* group in the first replicate, which resulted in 15 animals overall.

Starting in their 9th week and at 22 ± 1.6 kg live weight (LW), the animals were fattened on one of three different amino acid and energy balanced diets. The diets were applied considering three growth phases (Table 1) and two of the three were experimental diets where 50% in replicate 1 and 75% in replicate 2 of the soybean meal was substituted by either Spirulina (origin Myanmar) or partly-defatted *Hermetia illucens* larval meal (Hermetia Futtermittel GbR, Baruth/Mark, Germany).The first phase diets (25–50 kg LW) were fed for 5 (4, in second replicate) weeks, and the second phase for 3 weeks, totalling 8 (7) weeks until the animals achieved 75 kg LW. The last fattening phase of 5 (7) weeks included a 100% substitution of soybean meal. The third diet consisted of a typical pig diet, where the primary protein source was soybean meal; animals fed this diet make up the control group. The experimental and control diet components and analysed nutrient content for the three separate fattening periods are listed in Table 1.The diets were composed to demonstrate how the partial substitution of soybean meal by the alternative proteins affects the quantity of supplemented amino acids required to provide an amino acid supply similar to the soybean meal based control diet. In consequence, the diets differed markedly in the extent of amino acid supplementation. Information on the procurement and nutritional composition of the alternative protein sources can be found in Neumann, Velten, and Liebert (2017); basic nutritional information is also summarized in Table 2.

The animals were housed and fed in individual floor pens (2.75 × 1.25 m) outfitted with a feeding trough and nipple drinker at the Division Animal Nutrition Physiology, University of Göttingen, Germany and fed twice daily. Water supply was ad libitum. The room temperature was adjusted to the changing needs with age and was between 18 and 24 °C. The animals were kept on chopped straw and were weighed weekly. The second replicate animals were raised one week longer and weighed the day prior to slaughter, whereas for the first experimental replicate 5 days elapsed between weighing and slaughter for obtaining carcass yield. This study was approved by the Ethics Committee of the Lower Saxony Federal Office for Consumer Protection and Food Safety (LAVES), Germany according to article 4 of Germany's Animal Welfare Regulation (BMJV, 2001).

2.2. Slaughter and sample collection

With a final LW of 110.48 ± 5.1 kg resulting in a 90.96 ± 4.28 kg carcass weight (replicate 1) or 122.13 ± 6.3 kg resulting in a 99.71 ± 5.53 kg carcass weight (replicate 2), typical industry standard carcass weights (Statistisches Bundesamt, 2018), the animals were slaughtered by certified personal at the University of Göttingen slaughterhouse as authorized according to article 4 of the European Union's (EG) regulation No. 853/2004 (Regulation (EC) No 853/2004). The animals were slaughtered per diet group (HI, SP, C, respectively). The pigs were electrically stunned using a transformer and tongs system (Type LC1; Karl Schermer GmbH & Co.KG, Ettlingen, Germany) set to 220–230 V, 50/60 Hz and an amperage of 1.3. The animals were stunned using two applications: first 4 s to the head and then 4 s to the heart. The animals were then hung and bled for a minimum of 4 min prior to being scalded (60 °C–63 °C) and further cleaned and disembowelled. The carcasses were weighed post-slaughter and cooled at 4 °C overnight prior to butchering. The carcasses were classified according to lean meat yield per the manual 'Two Point' procedure (*Zwei-Punkt Messverfahren*) as the slaughterhouse was not equipped with automated or electronic classification equipment (Regulation (EU) 1308/2013). The thinnest portion of the fat layer on the *M. glutaeus medius* (GM) was used in later analysis as carcass fat parameter. The left *longissimus thoracis et lumborum* (LTL) was removed on the following day and cut into 2 cm thick steaks, which were systematically allocated for further analysis according to Fig. 1, resulting in the animal as the experimental unit of this study. Prior to trimming the LTL, a sample of subcutaneous adipose tissue (backfat) was procured from the cranial end. The backfat was used to monitor backfat colour and for fatty acid composition analysis.

The first two steaks were trimmed of excess subcutaneous fat and homogenised using a Grindomix GM200 (Retsch GmbH, Haan, Germany) that was cleaned between each sample. Then samples were frozen at −20 °C until meat composition analysis could be carried out. The third steak was physico-chemically evaluated as a means to determine baseline values for lean colour, lipid oxidation, cooking loss, and instrumental tenderness measurements. Three steaks from the midsection of the LTL were taken and separately stored at −20 °C until sensory testing was conducted. And the remaining anterior end of the LTL was used for the sensory training of panellists.

To consider how the alternatively-fed products behave under

Table 1
Ingredient (g/kg fed) and analysed nutrient (g/kg DM) composition of control (C), *Hermetia illucens* (HI) and Spirulina (SP) diets based on animal live weight (LW).

Animal LW	25–50 kg						51–75 kg						> 75 kg					
Ingredients/Diets	Replicate 1 (50%)			Replicate 2 (75%)			Replicate 1 (50%)			Replicate 2 (75%)			Replicate 1 (100%)			Replicate 2 (100%)		
	C	HI	SP	C	HI	S P	C	HI	SP	C	HI	SP	C	HI	SP	C	HI	SP
Wheat	365.2	369.5	371.8	365.0	371.2	376.0	394.1	397.1	399.0	394.1	398.9	402.2	416.8	427.1	431.1	416.8	426.6	430.8
Barley	365.2	369.5	371.8	365.0	371.2	376.0	394.1	397.1	399.0	394.1	398.9	402.2	416.8	427.1	431.1	416.8	426.6	430.8
Soybean meal	220.0	110.0	110.0	220.0	55.0	55.0	175.0	88.0	88.0	175.0	43.7	43.7	140.0	–	–	140.0	–	–
Soy oil	24.0	43.0	37.0	24.0	52.0	41.0	14.0	29.0	24.0	14.0	36.0	28.0	5.0	28.0	20.0	5.0	28.0	20.0
Premix[1]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
$CaCO_3$	11.0	10.0	10.0	11.0	10.0	10.0	9.0	9.0	9.0	9.0	9.0	9.0	8.0	7.0	7.0	8.0	7	7
NaCl	0.5	–	–	0.5	–	–	0.5	–	–	0.5	–	–	–	–	–	–	–	–
Hermetia	–	81.6	–	–	122.5	–	–	65.0	–	–	97.4	–	–	95.0	–	–	95.0	–
Spirulina	–	–	83.1	–	–	124.6	–	–	66.0	–	–	99.2	–	–	95.0	–	–	95.0
L-Lysine·HCl	3.4	4.8	5.4	3.3	5.1	6.0	3.0	4.1	4.6	3.0	4.4	5.1	3.1	4.8	5.5	3.1	4.8	5.5
DL-Methionine	0.1	0.7	0.4	0.7	0.8	0.4	0.4	0.2	–	–	0.3	–	–	0.3	–	–	0.2	–
L-Threonine	0.6	0.9	0.6	0.6	0.9	0.4	–	0.6	0.4	0.4	0.6	0.2	0.4	0.8	0.3	0.4	0.8	0.4
L-Leucine	–	–	–	–	1.3	–	–	–	–	–	0.7	–	–	–	–	–	1.1	–
L-Histidine	–	–	–	–	–	0.8	–	–	–	–	–	0.5	–	–	–	–	–	0.6
Analysed nutrients (g/kg DM)																		
Crude protein	190.4	217.1	192.4	197.0	198.1	191.0	201.4	234.2	203.2	181.4	181.4	185.2	138.9	172.0	172.7	170.0	161.1	149.6
Ether extract	50.3	89.1	65.3	50.9	94.3	70.5	42.0	75.1	60.6	41.5	82.60	60.30	36.3	71.2	51.5	32.6	65.6	45.9
Crude fibre	56.4	55.9	40.1	56.7	54.0	38.2	45.1	38.0	52.4	62.4	52.8	48.7	43.7	49.2	39.4	53.7	46.9	42.0
Crude ash	52.3	58.5	46.1	50.8	48.3	46.4	47.9	54.2	52.0	47.7	43.9	42.5	40.2	41.3	38.5	43.8	38.5	33.0
ME (MJ/kgDM)	15.3	16.1	16.1	15.3	16.4	16.4	15.2	15.8	15.8	15.2	16.1	16.0	15.1	16.0	15.9	15.1	16.0	15.9

[1] Supplementation of diets for growing pigs (per kg of final diet): Ca, 0.14%; P, 0.10%; Na, 0.12%; vitamin A, 4000 IU; vitamin D3, 500 IU; vitamin E, 40 mg; thiamine, 1.5 mg; riboflavin, 6.0 mg; vitamin B6, 3 mg; vitamin B12, 30 µg; vitamin K3, 3 mg; nicotinic acid, 20.0 mg; calcium pantothenate, 12.0 mg; folic acid, 0.5 mg; biotin, 100 µg; choline chloride, 100 mg; iron, 80 mg; copper, 5 mg; manganese, 27.5 mg; zinc, 75 mg; iodine, 0.68 mg; selenium, 0.2 mg; phytase (EC 3.1.3.8), 500 FTU.

Table 2
Analysed nutrient composition (% DM) of alternative protein sources Spirulina and partially defatted *Hermetia illucens* meal as used in diet formulation.

Nutrient content	*Hermetia illucens* Meal (partially-defatted)	Spirulina
Moisture (%)	5.5	3.4
Crude protein	60.8	58.8
Crude ash	7.5	6.1
Crude lipids	14.1	4.3
Crude fibre	10.9	0.5*

As documented in Neumann et al., 2017
* Preliminary data due to difficulties in application of the standard procedure.

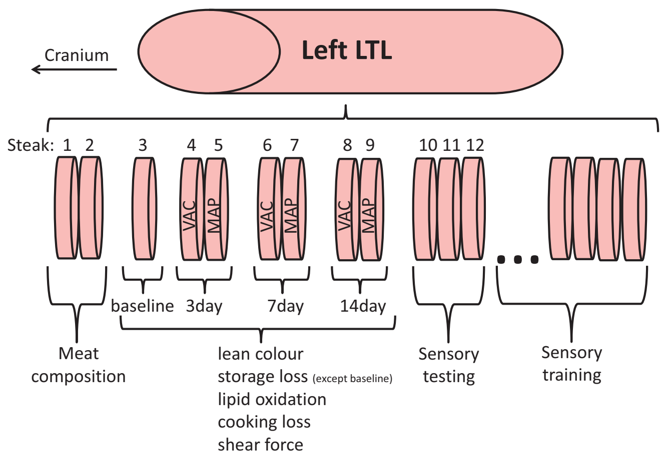

Fig. 1. Schematic representation of steak allocation. MAP refers to HiOxMAP and VAC refers to vacuum-sealed bag packaging systems. Baseline samples were not packaged.

HiOxMAP conditions compared to vacuum sealed bags, steak 4 to 9 were packaged in either of the packaging systems and stored at 4 °C without illumination for either 3, 7, or 14 days. Three steaks per animal allocated to HiOxMAP. These steaks (Steak 5, 7, 9; Fig. 1) were packaged in polypropylene (PP) plastic trays (227/178/40 mm in dimension) lined with a moisture absorbent pad, heat-sealed with a oriented polyethylene terephthalate (OPET)/polypropylene (PP) film (< 3 cm^3/m^2 24 h bar oxygen transmission rate; < 12 cm^3/m^2 24 h bar carbon dioxide transmission rate) and stored in a 80% O_2 / 20% CO_2 atmosphere. The remaining three steaks (Steak 4, 6, 8; Fig. 1) were vacuum-sealed in polyamide (PA) / polyethylene (PE) bags. After the allotted packaging time had elapsed, all samples were vacuum-sealed in polyamide (PA) /polyethylene (PE) bags and stored at −20 °C until further testing, with the exception of a 5 g piece of each steak that was separately packaged and stored at −80 °C to evaluate lipid oxidation.

2.3. Physico-chemical characteristics

The pH_{45min} was measured at 45 minutes post mortem in the LTL (between the 13th and 14th rib) as well as in the GM. On the following day, pH_u was measured only in the LTL. All measurements were taken with a portable pH meter equipped with a glass electrode and metal thermometer electrode (Knick Portamess 911, Berlin, Germany). The pH was measured by inserting the electrode completely into the muscle and an accompanying thermometer was inserted alongside the electrode approximately 1 cm away. Lean colour was measured on the exposed steak face prior to packaging (approximately 24 h post mortem; 5 minutes blooming time) and immediately after opening a package (no blooming time). The absence of blooming time for packaged samples was so that the colour would be as close to that perceived by the consumer looking through the packaging. CIELAB (L*a*b*) coordinate measurements were recorded and the values used in analysis were derived across the average of three measurements using a portable spectrophotometer (model: CM 600d, Konica Minolta, Tokyo, Japan) with a standard D65 illuminant. The spectrophotometer was calibrated using white and black objects provided by the manufacturer prior to every session. Delta L*a*b* values were calculated as values at package opening minus prior to packaging (24 hr post mortem) per steak. These measurements were used to assess the overall impact that packaging technology might have on colour over time. Meat composition parameters of steak 1 and 2 were analysed using a Foss FoodScan™ according to Anderson (2007). To determine storage loss, the steaks 4 through 9 were weighed prior to- and post-packaging; storage loss was expressed as the percent of weight loss over time compared to the initial sample weight. Cooking loss steaks were trimmed of exterior fat and cooked sous vide (separately vacuum-packaged) for 30 min in a heated water bath (Gesellschaft für Labortechnik mbH (GFL) Burgwedel, Germany) set to 73 °C to achieve a core temperature between 71 °C and 72 °C, as determined by a preliminary study. This core temperature was established so that the samples would achieve the same core temperature as those used for sensory testing. Cooking loss was expressed as the percentage of the initial weight. After determining cooking loss, the samples were stored at 4 °C overnight prior to instrumental tenderness measurements. Instrumental tenderness was determined according to Baublits, Meullenet, Sawyer, Mehaffey, and Saha (2006) using the MORS (razor blade) procedure with the following modifications: a TA.XTplus Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell was set to a penetration depth of 10 mm to penetrate approximately halfway into each sample. Each sample was sheared 6 times and further analysis was conducted with the mean value across the 6 measurements. Instrumental tenderness was measured as the highest peak (N). Finally, in order to determine the extent of lipid oxidation over time, the 2-thiobarbituric acid reactive substances (TBARS) method was conducted according to Bruna, Ordóñez, Fernández, Herranz, and De La Hoz (2001). Results were recorded in terms of µg of malondialdehyde (MDA)/g of sample.

2.4. Fatty acid composition of backfat

Fatty acid profiles were determined according to Liu, Trautmann, Wigger, Zhou, and Mörlein (2017) with the following modifications: a flame ionization detector (FID; Thermo Fischer Scientific, Waltham, Massachusetts, USA) heated to 260 °C was employed; the carrier gas helium flow was set to 1.2 ml/min; the run time was 20.17 min; and relative area data were analysed using Chromeleon Chromatography Data System (Version 7.2 SR4; Thermo Fischer Scientific, Waltham, Massachusetts, USA).

2.5. Sensory analysis

Conventional profiling was used to assign appropriate sensory attributes as well as evaluate the samples originating from the 47 animals. In eight 2 h training sessions, the assessors became familiar with pork chop products and developed a specific list of attributes to evaluate the pork samples of this study. The LTL end steaks (see Fig. 1) were used during training sessions. The 26 attributes covered appearance, odour, flavour, and texture. The final list included: overall odour, off-odour intensity, piggy odour, sour-like (lactic acid-like) odour, metallic odour, colour intensity (light-dark), rosiness (colour), overall flavour, off-flavour intensity, piggy flavour, brothy flavour, metallic flavour, sour taste, overall aftertaste, brothy aftertaste, sour aftertaste, metallic aftertaste, astringent aftertaste, hardness, juiciness, adhesiveness, malleability, moistness, crumbliness, tenderness, and the number of chews necessary prior to swallowing (see Table A.1 for more information). The pork samples were cooked as per the cooking loss method. The core temperature of every sample was tested (Testo 926 digital probe thermometer, Testo SE & Co. KGaA, Lenzkirch, Germany) prior to cutting and serving in order to ensure a minimum core temperature of 70 °C (for food safety purposes). The steaks were cut into approximately 1 cm cubes. The samples were served immediately on warmed plates and randomly assigned a 3 digit code. The assessor training and sample evaluations took place in the University of Göttingen sensory laboratory, which is in compliance with international standards (ISO, 2007).

The trained panel consisted of 10 assessors, who voluntarily provided written informed consent, were selected and trained according to ISO 8586-1 (1993) and were monetarily compensated for their time. In total, the panel evaluated the three products in question (Spirulina-fed pork, *Hermetia illucens*-fed pork, and conventionally (soy)-fed pork) in quadruplicate during four 1 h sessions. The assessors evaluated the samples in a sequential monadic order, where the sample orders were randomized and half of the panel received a different animal from the other half; therefore each animal was evaluated by 5 assessors. No assessor evaluated the same animal twice. The measurements were recorded electronically on 10 cm unmarked scales (number of chews was recorded as the absolute value), a point system from 0 to 100 was stored behind the scale for later statistical analysis, using EyeQuestion survey software (Logic8 BV, Elst, The Netherlands).

2.6. Statistical analysis

The variance analyses were carried out using SPSS (Version 25.0, IBM Corporation, Armonk, NY, USA) statistical software. Given that the aim of the study was to determine the overall effect of the protein source in a broad sense, the two replicates were pooled in the analysis and the differences in diet and duration were accounted for as the random replicate effect in the models. In addition, carcass weight was included as a covariate to additionally account for the varying weights between the two replicates. Therefore, mixed models were computed for baseline physico-chemical parameters and fatty acid composition, including diet (fixed effect) and replicate (random effect) with their interaction effect, as well as carcass weight as a covariate. The physico-chemical characteristics related to the duration and system of packaging were analysed using mixed models considering the diet and experimental replicate as between-subject factors and number of days packaged and type of packaging as the within-subject factors, where diet, package, storage and the associated interaction effects were set to be fixed effects, the replicate was a random effect, and carcass weight was included as a covariate. Sensory data were analysed using another mixed model with diet as a fixed effect, replicate, assessor and animal as a random effects, and carcass weight as a covariate. Throughout, a $p < .05$ threshold was used for variance analysis and Fischer's Least Significance Difference (LSD) test to determine statistical significance.

We computed a PCA, without rotation for semi-quantified fatty acids per animal, while including passive dummy variables for diets and fatty acid categories (SFA, MUFA, PUFA). The PCA input data were standardized so as to weight all fatty acids equally despite varying concentration levels, ie. omega 3 and 6 fatty acids are usually found in lower doses than most non-omega fatty acids, but are important distinguishing compounds. That is, PCA is based on correlations (as compared to co-variance when input data are not standardized). The PCA was computed using R (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria) coupled with the FactoMineR package (Lê, Josse, & Husson, 2008).

3. Results and discussion

3.1. Baseline and sensory pork quality

Overall, the dietary protein source had very little effect on baseline pork quality parameters, as well as sensory attributes. All physico-chemical parameters remained unaffected by diet (Table 3), except for the backfat lightness (L*). Nevertheless, the backfat L* values all remain within one point value of another and therefore are unlikely to be noticeable to the human eye. Further on the topic of L*, we must report that the lightness (L*) values of the meat samples are far above what would be expected in an industrial setting (Mörlein, Link, Werner, & Wicke, 2007), which could have implications on the overall physico-

Table 3
Estimated marginal means and standard error (in brackets) for baseline physico-chemical parameters based on the control (C), *Hermetia illucens* (HI) and Spirulina (SP) fed diets.

Parameter	C (n = 16)	HI (n = 15)	SP (n = 16)
Carcass weight (kg)[1]	95.08 (1.17)	97.99 (1.21)	93.11 (1.17)
Lean meat yield (%)[1]	59.52 (0.45)	58.77 (0.46)	59.05 (0.45)
GM pH_{45min}	6.08 (0.05)	6.24 (0.05)	5.97 (0.05)
LTL pH_{45min}	5.91 (0.08)	6.05 (0.09)	6.04 (0.09)
LTL pH_u	5.41 (0.02)	5.40 (0.03)	5.43 (0.03)
Cooking loss (%)	32.4 (0.30)	31.3 (0.30)	32.4 (0.30)
Instrumental tenderness (N)	10.78 (0.27)	10.49 (0.30)	10.51 (0.29)
Protein (%)	23.10 (0.12)	23.05 (0.13)	22.93 (0.13)
Intramuscular fat (%)	2.96 (0.19)	3.27 (0.21)	3.04 (0.20)
Water content (%)	72.60 (0.16)	72.02 (0.18)	72.44 (0.17)
Backfat L*	79.09^{a} (0.27)	78.17^{b} (0.30)	78.72^{ab} (0.29)
Backfat^{a}*	4.09 (0.18)	4.26 (0.19)	4.02 (0.19)
Backfat^{b}*	4.85 (0.20)	5.15 (0.22)	5.52 (0.21)
Lean colour L*	63.18 (0.89)	61.62 (0.97)	62.96 (0.93)
Lean colour^{a}*	2.74 (0.23)	3.56 (0.25)	2.94 (0.24)
Lean colour^{b}*	13.84 (0.30)	13.77 (0.33)	13.71 (0.32)
TBARS (µg/g)	0.357 (0.043)	0.399 (0.047)	0.470 (0.047)[2]

Superscript letters a-b denote statistical differences between groups at $p < .05$; no letters denote no identified significant differences.
[1] Computed without carcass weight as a covariate.
[2] n = 15.

chemical qualities observed in this study, such as water holding capacity (Joo, Kauffman, Kim, & Park, 1999). As for eating quality, diet only proved to have a statistically significant effect on three of the 26 sensory attributes tested (Table 4). Both Spirulina (SP) and *Hermetia illucens* (HI) protein sources resulted in stronger overall odours compared to the control group; whereas only HI resulted in significantly higher juiciness values compared to the control (and SP) group(s). This added juiciness is likely linked to the lower cooking losses as well as the fact that the HI product had the highest absolute, although not significant, IMF values, and could positively affect consumer preference towards such products (Aaslyng et al., 2007). Finally for astringent aftertaste, SP samples were evaluated as having a more astringent aftertaste compared to the control group, which could negatively affect eating quality. However, more research with other breeds or with fresh samples (ours were frozen) should be conducted to confirm this finding, given that the SP samples did not differ from HI samples and the HI samples did not differ from the control.

Finally, the basal diet ingredients should also be kept in mind as a possible influencing factor. The diets differed between the treatment groups with the aim to balance energy and amino acid ratios; however these adjustments led to the diets also differing in total fat and protein contents throughout the feeding phases. These inconsistencies likely influenced the growth as HI-fed animals were numerically heavier (+5 kg) than the SP-fed animals. However, these differences were not statistically significant.

3.2. *Storage and packaging effects*

As was to be expected, package type and storage time significantly affected product quality, as consistent with the literature (Bao & Ertbjerg, 2015; Delles, Xiong, True, Ao, & Dawson, 2014; McMillin, 2008; Spanos, Tørngren, Christensen, & Baron, 2016). In our study HiOxMAP packaging augmented lipid oxidation over time (Fig. 2), as consistent with Spanos, Tørngren, et al. (2016), who observed significantly higher TBARS values in LTL samples starting at 7 days of storage in modified packages with an atmosphere containing at least 50% oxygen.

Additionally, HiOxMAP increased instrumental tenderness values, ie. samples were tougher, (MAP M[1] = 13.74 ± 0.16 N vs. VAC M = 11.92 ± 0.17 N), as well as HiOxMAP increased L*a*b* values

Table 4
Estimated marginal means and standard error (in brackets) for statistically significant sensory attributes based on the control (C), *Hermetia illucens* (HI) and Spirulina (SP) fed diets; scale 0–100.

Parameter	C (n = 16)	HI (n = 15)	SP (n = 16)
Overall odour	62.3^{b} (3.2)	66.0^{a} (3.2)	66.3^{a} (3.2)
Astringent aftertaste	24.6^{b} (6.5)	28.1^{ab} (6.5)	31.1^{a} (6.5)
Juiciness	20.5^{b} (4.3)	25.6^{a} (4.3)	21.4^{b} (4.3)

Superscript letters a-b denote statistical differences between groups at $p < .05$.

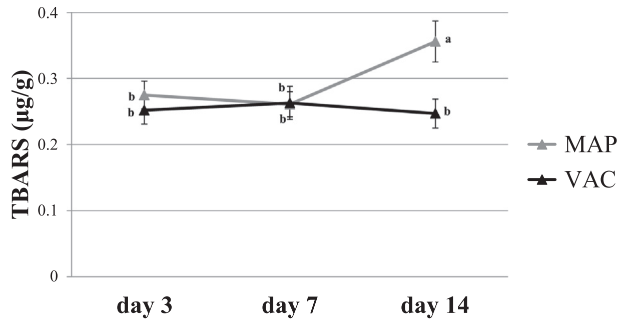

Fig. 2. Graphical depiction of TBARS estimated marginal means and standard error for highly oxygenated modified atmosphere (MAP) and vacuum-sealed bag (VAC) packaging over a storage time of up to 14 days. Superscript letters a-b signify significant differences between observations.

compared to VAC, but HiOxMAP a* and b* values eventually fell while VAC values increased, so that by day 14 values were within 1 point value (Fig. 3); although still above the measurements prior to packaging (zero on the y-axis).

Storage time also significantly affected meat product quality as expected (Fischer, 2007; Marcinkowska-lesiak et al., 2016; Pearce, Rosenvold, Andersen, & Hopkins, 2011; Spanos, Christensen, Tørngren, & Baron, 2016). In our study, storage losses (day 3 M = 8.81 ± 0.49 to day 7 M = 9.64 ± 0.36 to day 14 M = 10.53 ± 0.43) and cooking losses (day 3 M = 31.74 ± 0.16 to day 7 M = 33.00 ± 0.14 to day 14 M = 34.07 ± 0.15) increased over time. Instrumental tenderness was also affected by storage time (day 3 M = 11.41 ± 0.17 to day 7 M = 12.94 ± 0.17 to day 14 M = 14.12 ± 0.17), as samples lost more moisture over time.

Diet only had a rudimentary effect on product quality when packaged in different technologies over time; package type interacted with diet to minimally influence product characteristics. While packaged in HiOxMAP environments, soy- and Spirulina-fed samples exuded increased cooking losses compared to their counterparts in VAC packaging. However, HI-fed samples remained unaffected by HiOxMAP and on par with the other treatment groups in VAC packaging (Fig. 4). HiOxMAP packaging also increased the redness of all samples compared to VAC packaging, where samples a* decreased on average. Especially, HI samples were more intensively affected than SP samples (Fig. 5); however, HiOxMAP increased a* values relative prior to packaging (zero on the y-axis) whereas VAC decreased values.

3.3. *Fatty acid composition of backfat*

Diet significantly affected the fatty acid composition of the backfat (Table 5). The two alternative diets provided significantly higher polyunsaturated fatty acid (PUFA) contents; however the alternative protein sources affected different fatty acids to differing degrees. For example, the SP group is distinguished by omega 6 fatty acids and increased levels of C18:3n3 compared to the control group. As expected,

[1] Estimated marginal mean ± standard error

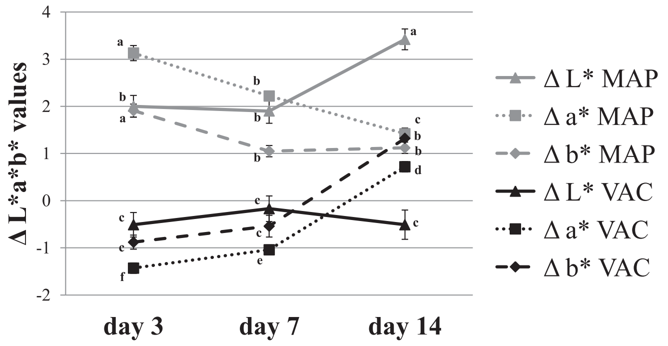

Fig. 3. Graphical depiction using estimated marginal means and standard error for highly oxygenated modified atmosphere (MAP) and vacuum-sealed bag (VAC) packaging over a storage time of up to 14 days and their effect on changes in lightness (L*), redness (a*), and yellowness (b*) relative to values prior to packaging (zero on y-axis). Superscript letters a-f signify significant differences between storage times and type of package for a specific colour parameter.

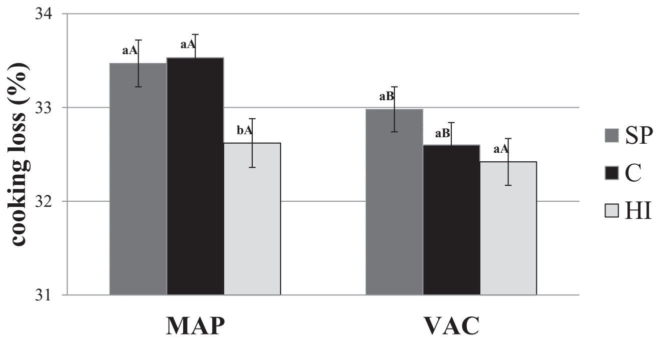

Fig. 4. Graphical depiction using estimated marginal means and standard error of cooking loss (%) for the interaction effect between packaging technology (highly oxygenated modified atmosphere packaging (MAP) vs. vacuum packaging (VAC)) and diet (spirulina (SP), *Hermetia illucens* (HI), and control (C)). Superscript lower case letters a-b signify significant differences between diets for each type of package and upper case letters A-B signify differences between the type of packaging for each diet.

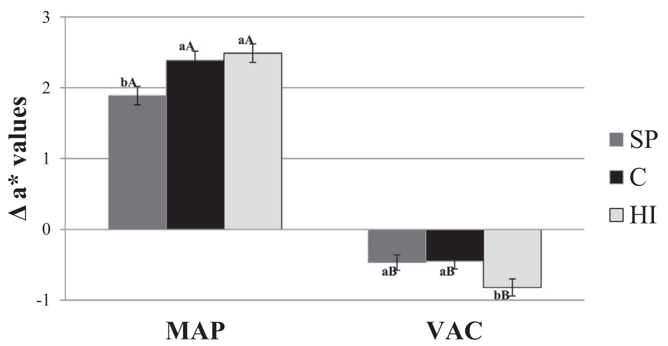

Fig. 5. Graphical depiction using estimated marginal means and standard error of the change in redness (a*) based on packaging technology (highly oxygenated modified atmosphere packaging (MAP) vs. vacuum packaging (VAC)) and diet (spirulina (SP), *Hermetia illucens* (HI), and control (C)). Superscript lower case letters a-b signify significant differences between diets for each type of package and upper case letters A-B signify differences between the type of packaging for each diet.

SP also increased the γ-linolenic acid (GLA; C18:3n6) content; yet the HI group was characterized also by superior GLA and C18:2 contents. Additionally, the HI group depicted an interesting mix of PUFAs and SFAs; lauric acid (C12:0) was five times higher in HI samples. The control was primarily characterized by the largest share of mono-unsaturated fatty acids (MUFAs). The three diet groups are distinguishable by their fatty acid patterns as to be seen from the PCA score plots (Fig. 6a). Although an overlapping of the control and Spirulina groups is evident, the overlapping is minimized when one apparent SP outlier (black square) is excluded from analysis. The HI group can be starkly differentiated from the other two based on the fatty acid profile. The PCA loading plot (Fig. 6b) depicts how the individual fatty acids contribute to that pattern. The validated PCA resulted in 6 components determined by screeplot visual interpretation; all 6 components contributed ≥5% explained variance; however the first (28.5% explained variance) and second (21.5%) components accounted for the most explained variance totalling 50%.

For the SP group, the result is not surprising, given that Spirulina is known to have a high PUFA content (Diraman et al., 2009), especially GLA (Diraman et al., 2009; Lang, Hodac, Friedl, & Feussner, 2011), which would likely be carried over into a monogastric product, such as pork (Juarez et al., 2016; Wood & Enser, 1997). Although the *Hermetia illucens* meal was partially defatted it still had a fat content 3 times higher than that of Spirulina, which likely contributed to its effect on the backfat fatty acid composition, especially the SFAs. In addition, the HI diet contained the highest fed soy oil levels, which may partially explain the superior PUFA content for the HI group as soybean oil is high in C18:2 (Shin et al., 2012). Nonetheless, *Hermetia illucens* larvae are known for their high saturated fatty acid (SFA) and usually low PUFA composition (Barroso et al., 2014; Spranghers et al., 2017) and as diet is usually responsible for contributing to n-6 fatty acids (Juarez et al., 2016), such as GLA, this finding was unexpected. However, the insect rearing substrate also plays an important role in determining the insect fatty acid composition and can affect the overall deposition of omega 3 and 6 fatty acids in insects (Spranghers et al., 2017). Furthermore, the high concentration of lauric acid appears well suited to being used as a biomarker (indicator) for HI-fed pork; however further research is needed to confirm the originality of this effect compared to other diet ingredients. Future research also needs to encompass the protein source influence on C22:5n3, which is found in high, yet differing, amounts in adipose tissue such as backfat (Alonso, Campo, Español, Roncalés, & Beltrán, 2009; Enser, Richardson, Wood, Gill, & Sheard, 2000) and not included in our study. In addition, a deeper look into the alternative protein sources' fatty acid compositions and their effect on meat products should be investigated in order to determine if such products are suitable to appeal to consumers in the future, i.e. such products could be marketed to health-conscious consumers (Kallas, Realini, & Gil, 2014).In addition, the technological properties of the fat tissue should be studied, e.g. its usefulness when producing fermented sausages or dry cured ham. Nonetheless, these findings provide first insights into the implications that such alternative protein sources have on monogastric-product fatty acid compositions.

Additionally, despite the differences in fatty acid composition amongst the three treatment groups, no associated adverse effects were exhibited under the industry's standard HiOxMAP packaging. Given the increased PUFA levels, increased TBARS values would be expected (Wood et al., 2004); however, in our study diet appeared to have no effect on lipid oxidation, even when packaged in an oxidation intensive atmosphere like HiOxMAP (Bao & Ertbjerg, 2015) and a possible anti-oxidative effect should investigated. On the other hand, both SP and HI products were reported to have a more intense overall odour. This could be attributed to the differing fatty acid composition (Wood et al., 2004); although this can be neither seen as a negative nor positive effect, as consumer perceptions and sensory preferences can be culturally dependent (Grunert, 1997; Sugimoto et al., 2016).

4. Conclusions

Pigs fed Spirulina or partially-defatted *Hermetia illucens* larval meal appeared to yield a raw meat product that is on par with soy-fed

Table 5
Estimated marginal means and standard error (in brackets) for fatty acid composition component relative area (%) [carcass weight as covariate].

Component(s) (%)	C (*n* = 16)	HI (*n* = 15)	SP (*n* = 16)
Saturated fatty acids (SFA)	40.37^{a} (0.44)	38.39 ^{b} (0.47)	39.24 ^{ab} (0.46)
C10:0	0.064 (0.002)	0.058 (0.002)	0.058 (0.002)
C11:0	0.003 (0.001)	0.000 (0.002)	0.006 (0.002)
C12:0	0.097^{b} (0.014)	0.551^{a} (0.015)	0.096^{b} (0.014)
C14:0	1.205^{b} (0.042)	2.150^{a} (0.046)	1.183^{b} (0.044)
C15:0	0.031 (0.003)	0.027 (0.003)	0.028 (0.003)
C16:0	25.22^{a} (0.25)	24.13^{b} (0.27)	24.67^{ab} (0.26)
C17:0	0.211 (0.017)	0.216 (0.019)	0.220 (0.018)
C18:0	13.34^{a} (0.23)	11.07^{b} (0.25)	12.78^{a} (0.24)
C20:0	0.154^{ab} (0.005)	0.142^{b} (0.005)	0.160^{a} (0.005)
C24:0	0.038 (0.002)	0.041 (0.002)	0.044 (0.002)
Monounsaturated fatty acids (MUFA)	43.89^{a} (0.42)	39.95 ^{b} (0.46)	40.95^{b} (0.44)
C14:1	0.013 (0.002)	0.031 (0.003)	0.014 (0.002)
C16:1	1.730 (0.046)	1.888 (0.050)	1.544 (0.048)
C17:1	0.187 (0.014)	0.124 (0.015)	0.159 (0.014)
C18:1n9c	37.98^{a} (0.39)	33.98^{c} (0.43)	35.64^{b} (0.41)
C18:1n9t	3.295^{a} (0.078)	3.396^{a} (0.085)	2.959^{b} (0.082)
C20:1n9	0.695 (0.025)	0.533 (0.027)	0.629 (0.026)
Polyunsaturated fatty acids (PUFA)	15.74^{c} (0.39)	21.66^{a} (0.40)	19.81^{b} (0.39)
C18:2n6	13.83^{c} (0.333)	19.15^{a} (0.362)	17.22^{b} (0.348)
C18:3n3	0.047^{b} (0.004)	0.050^{b} (0.005)	0.140^{a} (0.004)
C18:3n6 (GLA)	0.993^{c} (0.030)	1.535^{a} (0.033)	1.341^{b} (0.032)
C20:2n6	0.526 (0.015)	0.561 (0.016)	0.592 (0.016)
C20:3n3	0.144 (0.004)	0.170 (0.004)	0.169 (0.004)
C20:3n6	0.058^{b} (0.004)	0.058^{b} (0.004)	0.193^{a} (0.004)
C20:4n6	0.123 (0.005)	0.119 (0.005)	0.149 (0.005)
C22:6n3	0.011 (0.001)	0.011 (0.001)	0.011 (0.001)

Superscript letters a-c denote statistical differences between groups at $p < .05$; no letters denote no identified significant differences.

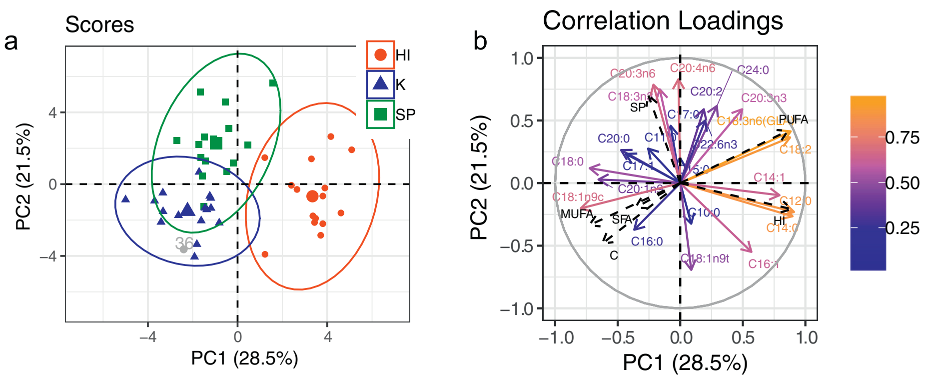

Fig. 6. Principal component analysis score (a) and loading (b) plots based on backfat fatty acid composition of spirulina (SP), *Hermetia illucens* (HI), and control (C) fed animals. One spirulina outlier was excluded in the analysis (black square; O).

animals. Experimental diets resulted in products that hardly differed in sensory aspects from the control, and differences found in alternatively-fed products could be interpreted as sensory improvements (i.e. more overall odour and increased juiciness). Furthermore, products rarely differed in physico-chemical parameters, even when packaged under industrial standard conditions. This means that the alternative diets could likely be integrated into the current production system with no adverse effects. In fact, given that diet had a significant effect on the backfat fatty acid composition, where both alternatively-fed groups had increased levels of polyunsaturated fatty acids, the alternatively-fed meat products should be further tested to ascertain if they could potentially be marketed at a premium and appeal to a broader range of consumers from a health conscious or sustainability point of view. These findings provided insights into how the search for more environmentally sustainable diets does not need to compromise meat quality.

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Appendix A

Table A.1
Sensory attributes identified and evaluated by trained assessors to characterize pork chops; scale from 0 to 100.

Attribute	Description	Sense	Reference(s)[1]
1. Overall odour	Intensity of all perceived odours	Smell	N/A
2. Off-odours	Intensity of all odours that do not belong to the product, including rancidity	Smell	N/A
3. Piggy odour	Intensity of pig or pork odour	Smell	N/A
4. Sour-like odour	Intensity of fermented product	Smell	100: Plain yogurt (lactic acid)
5. Metallic odour	Intensity of odour similar to blood or metal	Smell	Oxidized coins
6. Lightness	Intensity of the colour on the cut side	Visual	0: white paper
7. Rosiness	The direction of colour on the cut side	Visual	20: peach colour[2] 80: beige[2]
8. Overall flavour	Intensity of all perceived flavours	Taste	N/A
9. Off-flavours	Intensity of all flavours that do not belong to the product	Taste	N/A
10. Piggy flavour	Intensity of pig flavour specific to pork	Taste	N/A
11. Brothy flavour	Intensity of the all-rounded flavour like broth	Taste	N/A
12. Sour taste	Intensity of sour taste	Taste	50: 0 .4g/l citric acid solution
13. Metallic flavour	Intensity of taste similar to blood or serum	Taste	N/A
14. Overall aftertaste	Intensity of aftertaste after swallowing	Taste	N/A
15. Brothy aftertaste	Intensity of broth flavour left in mouth after swallowing, if at all	Taste	N/A
16. Sour aftertaste	Intensity of sour taste left in mouth after swallowing, if at all	Taste	100: Lemon juice (aftertaste)
17. Metallic aftertaste	Intensity of metallic flavour left in mouth after swallowing, if at all	Taste	N/A
18. Astringent aftertaste	Dry astringent feeling left in mouth after swallowing, if at all	Taste	100: 0 .2g/l Tannic acid solution (aftertaste)
19. Hardness	Strength (force) needed to bite through	Texture	0: spreadable cheese 50: gouda cheese cube 100: wine gummy
20. Juiciness	Amount of fluid released from product while chewing	Texture	0: Bread 50: Banana 100: Cucumber
21. Adhesiveness	Adhesive force felt between the teeth while chewing	Texture	0: Cucumber 50: dried fruit 100: Toffee
22. Malleability	Degree to which the sample is deformed while chewing	Texture	100: chewing gum
23. Moistness	Degree of moisture felt on the sample while chewing	Texture	
24. Crumbliness	Number of particles perceived in mouth directly prior to swallowing	Texture	0: water solution 20: apple sauce 100: chewed carrot
25. Tenderness	Amount of strength needed to chew sample	Texture	30: ripe apricot 100: dried beef
26. Number of chews	Number of chews required prior to swallowing	Texture	Absolute number recorded

[1] Values represent approximate placement on training scales.
[2] colour cards specific to the tested products were customized while the list of attributes was established.

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Paper 4: Consumer Acceptance of Alternative Animal Feeds: Effects of Meat Color and Information

In preparation for submission to *Food Policy*

Abstract: Growing global demand for animal-based proteins requires an increase in production as well as sources of protein feedstuffs. Using a discrete choice experiment with chicken breast, including an information treatment about production conditions and meat quality aspects, we elicit consumer preferences for two alternative feedstuffs, spirulina and insect meal. Spirulina results in a dark orange-red meat color when incorporated into poultry diets; insect meal intensifies yellow-hues. Uninformed consumers reject chicken breast produced with spirulina; however, the yellow appearance of chicken breast produced with insect meal is preferred irrespective of information. Informed consumers are willing to accept, but do not prefer chicken breast produced with spirulina. With information and a declaration, consumers highly prefer insect meal as a feedstuff. Information on feedstuffs leads to heterogeneous preferences; including a psychometric scale into the model shows that positive preferences are derived from environmental consciousness; however, there remains a portion of consumers who are disgusted by insect meal as a feedstuff. In conclusion, consumers may be willing to accept chicken breast produced with alternative protein feedstuffs. It is vital that credible information and labelling are included in the process; thus raising questions over the need for the mandatory declaration of feedstuffs in meat production.

Consumer acceptance of alternative animal feeds: effects of meat color and information

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Abstract: Growing global demand for animal-based proteins requires an increase in production as well as sources of protein feedstuffs. Using a discrete choice experiment with chicken breast, including an information treatment about production conditions and meat quality aspects, we elicit consumer preferences for two alternative feedstuffs, spirulina and insect meal. Spirulina results in a dark orange-red meat color when incorporated into poultry diets; insect meal intensifies yellow-hues. Uninformed consumers reject chicken breast produced with spirulina; however, the yellow appearance of chicken breast produced with insect meal is preferred irrespective of information. Informed consumers are willing to accept, but do not prefer chicken breast produced with spirulina. With information and a declaration, consumers highly prefer insect meal as a feedstuff. Information on feedstuffs leads to heterogeneous preferences; including a psychometric scale into the model shows that positive preferences are derived from environmental consciousness; however, there remains a portion of consumers who are disgusted by insect meal as a feedstuff. In conclusion, consumers may be willing to accept chicken breast produced with alternative protein feedstuffs. It is vital that credible information and labelling are included in the process; thus raising questions over the need for the mandatory declaration of feedstuffs in meat production.

Keywords: meat color, insect meal, spirulina, labelling, information treatment

1. Introduction

A growing global population receiving higher incomes is increasing the demand for animal-based protein (FAO, 2015). This trend in global diets necessitates increasing amounts of plant-based protein as feed inputs into intensive livestock production systems. Even when considering mitigating measures, such as vegetarian and vegan diets, the future supply of protein feedstuffs will need to be independent of arable land in order to avoid further changes in land use (Röös et al., 2017). Changes in land use that are required to produce more meat protein are non-reconcilable with current environmental and sustainability goals; and is a critical limitation to the future supply of meat from monogastric animals, such as poultry, that rely on high quality protein feedstuffs.

Although the gap between the demand for protein feedstuffs and its supply is a global one, coined the *protein gap*, the problem is particularly exacerbated in specific regions. For example, Western European poultry and swine production are heavily reliant on imports of soybean meal (Schreuder and De Visser, 2014); and makes producers vulnerable to changes in the world soy market (Song et al., 2009) and its powerful leading suppliers. Furthermore, European consumers are becoming increasingly aware of European agriculture's dependence on imported soy for livestock feed and are demanding changes to current practices in light of the negative environmental externalities of soybean production. This compounds with the fact that these consumers are skeptical of genetically modified crops (Christoph et al., 2008), including those used as feedstuffs (Profeta and Hamm, 2019a), of which soybeans is the most well-known case. The controversies surrounding soybean imports has led to consumer preferences for animal products raised with locally grown feedstuffs (Profeta and Hamm, 2019b).

Efforts to decouple the production of protein feedstuffs from land use have focused on non-traditional sources of protein including microalgae, and insects. The microalga spirulina (*Arthrospira platensis*) and insect meal derived from the larvae of the black soldier fly (*Hermetia illucens*) have been verified as viable replacements of soybean meal in the diets of boiler chickens by animal nutritionists (Neumann et al., 2018). Both species feature favorably high levels of crude protein (Tokusoglu and Unal, 2003) and can be cultivated in bioreactors on a range of waste material sources that reduce costs and increase the overall sustainability of production (Diener et al., 2011; Hultberg et al., 2017; Olguín et al., 2003; Oonincx et al., 2015; Spranghers et al., 2017).

However, while research on production and processing and use of insects as feedstuffs are well underway, legislation and regulations of insect feed farming are now only unfolding in many countries. Legal barriers to allow the use of insects as animal feed were first lifted by the US Food and Drug Administration (US FDA) and the Canadian Food Inspection Agency (CFIA) in 2016 with the approval of black soldier fly products for salmon and poultry feed, respectively (Lähteenmäki-Uutela et al., 2017).

The use of insect proteins for feed production in the European Union (EU) is hindered by two main pieces of legislation. Regulation No 999/2001 (2001), commonly referred to as the 'feed ban', prohibits the use of animal-derived protein in feedstuffs in the aftermath of the BSE crisis. In 2016, the European Commission introduced an amended Regulation 999/2001 which authorizes the use of insect processed animal proteins (PAPs) in feed for aquaculture animals since 2017 (EC 2017/893, 2017). A second constraining factor concerns the use of source materials as feed in the production of insects. The EU regulation on animal by-products (Regulation No 1069/2009, 2009) defines insects produced for feed purposes as 'farmed animals', which greatly limits the means by which insects can be fed. Waste slurry, manure, food waste or unprocessed foodstuffs containing meat or fish are prohibited. Moreover, the insect production environment itself must adhere to stringent EU food safety and hygiene standards that are governed by the EU's 'novel food' legislation (EC 2015/2283, 2015).

As both consumers and policy makers across many markets are keen to advance alternative sustainable food systems, research into current knowledge gaps and related challenges is essential for developing regulatory and market environments that maximize social welfare. Such an advancement in alternative sustainable food systems is paramount in fulling the Sustainable Development Goals laid out by the United Nations. In this paper, we argue that understanding consumer acceptance and willingness to pay (WTP) for meat produced with alternative novel feedstuffs is fundamental to overcoming the current regulatory uncertainty regarding insect-based feedstuffs in the EU and to guide the marketing and adequate labelling of consumer products, ensuring their successful integration into a sustainable food system.

Therefore, the study captured in this paper evaluates consumer acceptance of poultry products produced with novel feedstuffs containing spirulina and soldier fly based protein. A series of discrete choice experiment were conducted with chicken breast produced with the respective feedstuffs and information treatments, which identified and quantified consumer preferences and their heterogeneity of raw meat colour, information on the composition of poultry feed, and labelled health and environmental claims.

2. Literature

Consumer acceptance is crucial to the success of food product and food technology innovations alike (Siegrist, 2008). Yet, despite an apparent increase in interest in insect-based foods (entomophagy) and those produced with alternative feedstuffs (e.g. algae), the ultimate future success of insects or algae as food and feed hinges on consumers' behavioral response in the marketplace. The innovation literature ascribes perpetually high rates of food product innovation failure to widespread consumer resistance to innovation (Garcia et al., 2007) due to either functional and/or psychological barriers.

In response to the growing attention paid to insects as alternative protein sources, the majority of existing literature has investigated several sociocultural and psychological barriers to insects as food in non-traditional western consumer markets (Hartmann et al., 2015; Mancini et al., 2019). Several studies find consumers unwilling to accept insects as food, citing disgust and food neophobia, the reluctance to eat unfamiliar foods (Gere et al., 2017; Hartmann et al., 2015; La Barbera et al., 2018; Laureati et al., 2016; Verbeke, 2015), followed by product availability (House, 2016; Menozzi et al., 2017) and price (House, 2016) as leading factors in consumer rejection. Especially, affective factors, including disgust, have been shown to explain consumer likelihood to try and pay for novel insect-based products (Onwezen et al., 2019; Powell et al., 2019). Consumer willingness to consider eating insect-based foods increases when familiar foods contain no visible trace of insects (Barsics et al., 2017; Caparros Megido et al., 2014; Delicato et al., 2020; Hartmann et al., 2015; Schouteten et al., 2016; Tan et al., 2017; Verbeke, 2015); with insects as feed being one of the most promising strategies for incorporating insects into the food systems while retaining consumer acceptance (Marberg et al., 2017; Onwezen et al., 2019). Another stream of studies (Hartmann et al., 2018; Kostecka et al., 2017; Verbeke, 2015) indicate that the provision of information about the positive environmental effects of insect- or algae-based foods and their sustainability attributes has the potential to shift consumers' acceptance. Several studies point to positive effects of information about the beneficial properties of edible insects for sustainability on consumers' willingness to consume insect-based products (Laureati et al., 2016; Lensvelt and

Steenbekkers, 2014; Verbeke, 2015). However, such utilitarian information strategies may be of limited effectiveness compared to hedonic messaging that nudges skeptical Western consumers with positively accepted information on sensory attributes instead of attempting to rationalizing "disgust" away (Berger et al., 2018; Deroy et al., 2015).

As consumers of meat have become increasingly more interested in the way their food is produced, the literature on the economics of information and labeling to consumers has addressed a wide ranging set of issues, including nutrition and health (e.g., Variyam, 2008), "free from" (e.g. Lusk et al., 2003), animal welfare (e.g. Tonsor and Wolf, 2011), the sustainability of livestock systems (e.g., Grebitus et al., 2013b; Peschel et al., 2016; Risius and Hamm, 2017; Van Loo et al., 2014), and recently aspects regarding feedstuffs used in production (Profeta and Hamm, 2019b). However, the ability of proliferating label systems conveying increasingly redundant information to enable consumers to make better food choice decisions is increasingly put into question (Bernard et al., 2019; Bonroy and Constantatos, 2015; Messer et al., 2017).

The positive effects of information provision on consumer decisions making and WTP are well documented in the literature. However, several studies (e.g., Bronnmann and Asche, 2017; Grebitus et al., 2013a; Ochs et al., 2019; Risius and Hamm, 2017) suggest that more and/or new information on product or process attributes, including animal production system, packaging technology, and meat color may amplify underlying preference heterogeneity in a consumer population. While investigating the sources of preference heterogeneity for complex credence attributes, the consumer choice literature increasingly employs psychometric measures including attitudinal scales regarding food neophobia (Chen et al., 2013; La Barbera et al., 2018), or the environmentally oriented new ecological paradigm (NEP) scale (Steiner et al., 2017) to elicit latent consumer cognitive factors in food choice decisions. In the context of psychometric influences, the extent to which information builds consumer trust by converting credence attributes of insect-based food into quasi-searchable information may be especially important to engage with young European consumers who appear to be most ready to accept such foods (Schouteten et al., 2016; Verbeke, 2015). The broader question whether meat produced with insect- or algae based ingredients needs to carry pertinent information in the form of (feed) ingredient declaration, or otherwise, is unmistakably answered in current regulations in the EU, Canada and the US alike (Lähteenmäki-Uutela et al., 2017; Marberg et al., 2017).Yet, despite the growing interest in the topic, studies are still lacking, which investigate pathways for overcoming the widespread rejection particularly of insects as food or feed and especially those that inform policy uncertainty regarding effective labeling and information strategies (Lombardi et al., 2019).

Known as one of the most important search attributes influencing consumer meat choices, fresh meat (poultry) color is a familiar indicator of freshness (Droval et al., 2012). Despite its importance in the marketplace, however, few studies quantify consumer preferences and acceptance of meat color (Grebitus et al., 2013b; Lusk et al., 2018; Ngapo et al., 2007; Viana et al., 2005); especially in poultry. The literature agrees in that consumer preferences for pork or beef color vary as associated with quality grades (Lusk et al., 2018), packaging (Grebitus et al., 2013b; Viana et al., 2005) and underlying consumer characteristics (Ngapo et al., 2007), suggesting that acceptance thresholds likely exist for different consumer groups. Yet, evidence of consumer preferences for poultry meat color is scarce. A review of literature by Wideman et al. (2016) suggests that besides processing and chemical influences (e.g. bleaching), feed (e.g. wheat, corn) is major determinant of poultry meat color. Despite being labelled as "white meat", the color of poultry meat evolves in red/pink and yellow tones. Kennedy et al. (2005, 2004) are among a few to investigate consumer expectations of poultry meat appearance and describe consumers as expecting a "characteristic pink color" of chicken breast, whereas a "yellowish" coloring reduces the overall liking of chicken breast among UK consumers. The meat science literature (e.g., Altmann et al., 2020; Toyomizu et al., 2001; Venkataraman et al., 1994) suggests that the use of insect- or algae-based poultry feedstuffs (e.g. spirulina, black soldier fly larvae) intensifies red and/or yellow hues in raw poultry meat. Overall, the somewhat limited and ambiguous evidence regarding consumer acceptance of poultry meat color suggests that meat color is likely to play a significant role in consumer response to alternatively fed poultry meat products.

Overall, it is clear that potential consumers of meat produced with novel feedstuffs may face widespread asymmetries and lack of information regarding unfamiliar product and/or process attributes, as well as underlying policies and emerging regulations that govern insect and algae production and their use as food or feeds. Therefore, it is important to answer the question of how consumers will behave in a non-hypothetical market when asked to express their preferences for meat products produced without soybean meal, instead containing novel and alternative protein feedstuffs derived from the microalga spirulina or black soldier fly. Furthermore, how product information and labelling affect consumers' choice decisions and determinants of WTP is of relevance. Finally, it remains to be seen what a deeper understanding of consumer acceptance and choices holds for food policy and the regulation of (feed) ingredient labeling as well as marketing of novel insect and algae-based animal feedstuffs in major consumer markets.

This paper contributes to the literature on consumer preferences for sustainable innovation in livestock production systems by providing the above research questions. Our particular focus lies on advancing the understanding of individual preferences for unfamiliar product color, as well as how information affects individual's evaluation of meat resulting from animals being fed a diet containing insect- or and algae-based protein. Moreover, we empirically test whether product labelling that emphasizes positive environmental benefits (e.g. replacing soy meal) and a health claim (e.g. elevated omega 3 from algae-based feed) adjacent to the credence attribute "fed with …" is able to override individual's concerns regarding meat color or their potential disgust of insect meal as feed. To the best of our knowledge, this paper is the first to estimate consumer preferences and willingness to pay for novel insect- and algae-based meat products through a hypothetical WTP elicitation mechanism that considers the effectiveness of alternative channels and types of information on consumer acceptance and preference.

3 Material and Methods

In order to systematically elicit consumer preferences or readiness for insect- or algae-based feedstuffs, a quantitative consumer survey was compiled.

Description of survey

The survey included indirect and direct methodological approaches. The direct survey portions included sociodemographics, general consumer shopping behavior as well as attitudes towards 'green' shopping. The indirect part included choice behavior elicited using a discrete choice model, including the attributes of feedstuff, labelling, and price; an information treatment split the sample.

Choice experimental (CE) methods have become the workhorse for eliciting individuals' preferences in a systematic, nevertheless, indirect manner (Louviere et al., 2000). This allows a closer look at how consumers behave in complex decision-making situations and how they react to, process, and use information provided to them (Reisch and Zhao, 2017).

Following neoclassical theory and cognitive learning theory, it can be assumed that individuals process and use all available information as part of a fully informed, rational decision. However, the context of novel protein feedstuffs in meat production resulting in unfamiliar meat color and the widespread disgust for insect-based foods among consumers gives reason to assume that the process of delivery of information and its interaction with other experimental attributes play a key role in consumer acceptance and WTP.

A second component of the indirect test assessment, psychometric scales used to assess consumer readiness and liking of new food technologies and health as well as environmental values, has been included to explain consumer heterogeneity in this. The Food Technology Neophobia Scale (FTNS) (Cox and Evans, 2008), The New Ecological Paradigm (NEP) Scale (Dunlap et al., 2000) and the Wellness Scale (Kraft and Goodell, 1993) were included to identify neophobic, environmental- and health-conscious consumers, respectively. FTNS has been found to be useful in explaining consumer behavior towards meat color and packaging technology (Chen et al., 2013). Similarly, NEP has been shown to be useful in unveiling consumer values regarding the environment (Steiner et al., 2017) to further assist in explaining consumer motivations. To the best of our knowledge, the validated Wellness Scale has not yet been used to explain consumer motivations for food, but is more commonly applied in public health and medical fields. The three scales were chosen to be the most likely psychometric aspects for explaining individual consumer choice behavior. Ultimately, NEP proved best able to explain the variation in choices and was included in further analysis.

Operationalization of the survey

Both, indirect and direct survey parts were programmed in a consumer survey questionnaire mask (Unipark, Questback GmbH, Cologne, Germany). Ethical approval for this study was granted by the University of Goettingen Ethics Committee prior to data collection.

The choice experiment was created using standardized photos taken by the first author of three different chicken breast packages. The photos were edited to include a sales tag with price per package and kilogram, as it is obligatory in Germany (Figure 1). Package and kilogram prices were based on a market price check (covering two federal states) conducted in the fall of 2017, right before the survey. At least one location per major grocery store chain was visited in each state to ascertain the price range for non-organic chicken breast. In the end, prices of 2.99 €, 5.98 € and 8.98 € per package were used in the experiment. In addition, labels signifying sustainability

(ProPlanet) and healthiness (rich in omega fatty acids) were included in the design. ProPlanet is a third party label used by one of the leading retail groups in Germany to assign general combats for sustainability: ecological, societal, and economic aspects (http://www.proplanet-label.com/). We included ProPlanet because of its broad sustainability claim, as well as it is one of the few sustainability labels currently found on meat products in Germany. Attributes and levels included in the design are listed in Table 1. The design was an orthogonal design, created by SPSS Version 22, with randomly allocated choice cards. Subsequently, respondents completed a total of 9 choice sets consisting of two different chicken breast products and a ‘no buy’ option.

kein Kauf translates to ‘no buy’ and *gefüttert mit Soja/Spirulina* translate to ‘fed with soy/spirulina’

Figure 1: Example choice set as shown to informed respondents.

Table 1: Attributes and levels applied in the choice experiment

Attribute	Levels
Feedstuff	Spirulina (SP) Insect (IN) Soy
Label	ProPlanet (PRO) Rich in omega fatty acids (OMEGA) No label
Price	2.99 € (5.98 €/kg) 5.98 € (11.96 €/kg) 8.98 € (17.96 €/kg)

The survey was distributed by Survey Sampling International (SSI) to a German-wide online panel in February 2018. Respondents were randomly selected based on gender and age quotas representative of census data (Statistisches Bundesamt, 2017) and only household shoppers of chicken breast were included. A total of n=1197 respondents completed the survey. The data were then checked to ensure quality based on three trap questions and duration to complete the survey. A trap question consisted of two statements per attitude scale; where answers to one pair of attitude statements was already given and thus obvious, e.g. “plants and animals have as much right as humans to exist” (now click: strongly agree) and “humans were meant to rule over the rest of nature” (now click strongly agree), respondents were discarded. Respondents with completion times less than 5 minutes were also discarded based on a natural break in the completion times as well as pre-testing showed approx. 10 minutes was required to thoroughly read and answer each question. After quality checks, 1074 responses remained, split as uninformed (n=540) and informed (n=534) respondents. The final sample and split-groups provided a reasonable data, replicating census data; deviations include slightly decreased overall household incomes and a higher proportion of the highly educated (university degree holders) (Table A1).

Description of Information treatment

Approximately half of the sample completed the discrete choice experiment after receiving written information on the specific protein feedstuffs used in chicken breast production; the chicken breast packages were also indicated as, e.g., 'spirulina-fed' throughout the choice experiment. Information per protein feedstuff was listed on a mandatory screen, where consumers could not click continue until 30 seconds had elapsed. In total, three screens were shown corresponding to the protein feedstuffs as outlines in Table 2. The remainder of respondents completed the choice experiment without information or knowledge of which feedstuffs were used during production.

Table 2: Information statements provided to the split sample

Protein Feedstuff	Information Treatment
Spirulina	▪ Spirulina-fed poultry produces meat with a high content of omega fatty acids ▪ Spirulina is regarded as a "super food" ▪ The use of spirulina in the feeding of poultry can reduce the area required for agricultural feed production ▪ Spirulina, which contains carotenoids, gives poultry meat a light reddish-orange color
Insect	▪ Insects are a naturally eaten by poultry ▪ Insect-based feed can increase the sustainability of poultry production ▪ Insect-based feed can increase the flavour of poultry meat
Soy	▪ Soy is the most important protein feed in German poultry production ▪ The cultivation of soybeans has driven the deforestation of rainforests in South America ▪ Soy has a protein composition that is important for the growth of poultry ▪ Feeding high amounts of soy can lead to an accumulation of omega-3 fatty acids in poultry meat

Econometric Models

For many years, the multinomial logit model (McFadden, 1973) was the benchmark for analyzing choice data. This model assumes homogenous consumer preferences. Therefore, an extension of the model was presented – the random parameter logit model (RPL) by Revelt and Train (1998). A RPL model is better equipped for repeated choices, as in our experiment, as it allows heterogeneity of preferences through the assumption of at least some random parameters, following a certain continuous probability distribution.

Brownstone and Train (1999) and McFadden and Train (2000) show that RPL models can approximate any random utility model arbitrarily well, provided the researcher uses the correct mixing distribution. Hess and Train (2017) explore this potential by specifying the most general form of a mixed logit model in which all utility coefficients are randomly distributed and estimate a full covariance matrix among them. However, one of the main drawbacks of estimating RPL and RPL-EC models is that the correct specification of the random parameters and their distribution may involve a long trial-and-error process. The same holds for the EC structure in the absence of a priori expectations regarding substitution patterns (Walker et al., 2007). A further warning is that the correlation of random parameters is introduced through Cholesky factorization, which is sensitive to the order of the variables (Henscher et al., 2005).

Within, it is assumed that a portion of the utility is deterministic (based on attributes) and the remainder is unobservable. The utility received by individual i from alternative j (in our study a package of chicken breast) given choice scenario t is explained by

$$U_{ijt} = \beta_i X_{ijt} + \varepsilon_{ijt},$$

where ε_{ijt} is the random unknown component across individuals for j alternatives during t. The deterministic portion comprises X_{ijt}, which is a vector of j's observable attributes, and β_i as a vector of unobserved coefficients pertaining to the heterogeneous product preferences across the individuals, but not depending on alternatives.

The deterministic portion is assumed to be linear, so that individual i's marginal utilities of observable attributes (h = price, feedstuff, etc.) associated with alternative j can be summed with a positive scale factor σ:

$$U_{ijt} = \sigma \sum_{h=1}^{H} \beta_{ih} X_{ijth} + \varepsilon_{ijt}.$$

where β_{ih} is the marginal utility received by individual i from attribute h, estimated by:

$$\beta_{ih} = \bar{\beta}_h + i_h z_{ih},$$

where $\bar{\beta}_h$ is the mean of marginal utilities derived from the sampled population, i_h is the deviation of preferences among individuals and z_{ih} represents the random draws prescribed from a pre-specified distribution for individual i and attribute h.

Following utility theory, an individual is always trying to maximize their utility; therefore, the probability of individual i choosing alternative j during choice scenario t is

$$P_{ijt} = P\left(U_{ijt} > U_{ikt} \ \ \forall k \ \neq j\right).$$

We estimated our RPL models with 2000 Halton draws. We coded feedstuff and label attributes as dummy variables, as well as the no buy option. Reference levels for feedstuff and label attributes were 'soy', because it is currently the industry standard and 'no label' in order to determine marginal utility and its respective WTP. Price was input as a continuous variable. Feedstuff and label attributes were modelled as random components in RPL models. All others were input as fixed variables. Hence;

$$U_i = \ \beta_i price + \beta_i SP + \ \beta_i IN + \beta_i PRO + \beta_i OMEGA + \ \beta_i NOBUY$$

Since we can assume that there maybe correlation patterns with regard to the unveiled choices, we employed the extension (RPL-EC), which accounts for correlation for between the preferences for the attributes under study (Scarpa et al., 2007).

Using attitudinal scale data (NEP value) we included an interaction effect in our model, while holding all other variable conditions as described above. Incorporating interactions is a common approach to account for observable consumer preference heterogeneity (Ding et al., 2015). Our NEP interaction model can be represented as

$$U_i = \ \beta_i price + \beta_i SP + \ \beta_i IN + \beta_i PRO + \beta_i OMEGA + \ \beta_i SP * NEP + \ \beta_i IN * NEP + \ \beta_i PRO * NEP + \ \beta_i OMEGA * NEP + \ \beta_i NOBUY$$

where SP signifies feedstuff; IN signifies insects as a feedstuff; PRO pertains to the ProPlanet label; OMEGA corresponds to the rich on omega fatty acids label; and the parallel terms with NEP are continuous variables relating to a positive choice of attribute *h* by individual *i* and his/her associated NEP value.

Finally, we estimated WTP in preference space for the feedstuff and labels for all RPL models according to Henscher et al. (2005):

$$WTP = -\ \frac{\beta_{attribute}}{\beta_{price}}$$

Uninformed and informed consumer data were analysed with the same model specifications, however, different data sets (informed vs. uninformed).

4.0 Results and Discussion:

Our models (Table 3) inform consumer acceptance of chicken breast raised using alternative feeds given two specific scenarios: 1) industry were to passively integrate spirulina or insect meal into their production without informing consumers; 2) consumers are actively informed regarding feed ingredients used in production of chicken breast and the resulting products are labelled.

In the first case, consumer preferences largely rely on meat appearance (color) and/or labelling claims. Consumer acceptance for an orange-red raw meat color is depicted by the SP mean coefficient, which remains negative across uninformed consumer models; although only the standard model produces a statistically significant coefficient. Our results complement the work of Kennedy et al. (2005, 2004) in exploring the boundaries of consumer acceptance for chicken breast meat color, as well as Lusk et al. (2018) and Grebitus et al. (2013b) in indicating meat color's significant role in determining willingness-to-pay for meat products. The negative mean coefficients correspond with large and highly significant standard deviation coefficients, illustrating that the raw meat color affects consumer preferences heterogeneously. The deviation from more common pale (Wideman et al., 2016), pink (Kennedy et al., 2004), or yellow (Williams, 1992) colors usually preferred for chicken breast, i.e. darker more orange color, likely affects consumers based on multiple factors. Some consumers may be wary of the unfamiliar color; whereas others may not include raw meat color as an important choice criterion (Lusk et al., 2018).

Surprisingly, there is a clear consumer preference, based on appearance, for the chicken breast produced with insect meal. This is illustrated by positive and significant IN mean coefficients for uninformed models; standard deviations remain insignificant. Unlike with Kennedy et al.'s (2005) UK consumers, our models indicate that the German population prefers a yellow-ish chicken breast, as Williams (1992) expressed for USA consumers. This finding underlines persisting geographical differences in meat color preferences (Ngapo et al., 2007), which should be exploited (not under-estimated) in the globalized meat industry.

Table 3: RPL model correlated error component (RPL-EC) estimates of coefficient means and standard deviations (SD) for uninformed and informed treatment groups using

Coefficients	**Uninformed (n= 540)**				**Informed (n=534)**			
	Standard		**NEP Interaction**		**Standard**		**NEP Interaction**	
Mean	*Coefficient*	*St. error*	*Coefficient*	*St. error*	*Coefficient*	*St. error*	*Coefficient*	*St. error*
Non-random parameters								
Price	-0.36***	0.01	-0.36***	0.01	-0.35***	0.01	-0.35***	0.01
NOBUY	-2.71***	0.15	-2.73***	0.15	-2.05***	0.23	-2.06***	0.11
SP*NEP			-0.03	0.24			0.56***	0.17
IN*NEP			-0.14	0.14			0.95***	0.20
PRO*NEP			0.40***	0.14			-0.00	0.12
OMEGA*NEP			0.09	0.13			-0.24	0.16
Random parameters								
SP	-0.36***	0.12	-0.23	0.92	0.16	0.13	-1.90***	0.70
IN	0.36***	0.08	0.91*	0.52	0.83***	0.13	-2.69***	0.76
PRO	0.92***	0.09	-0.59	0.55	0.33***	0.07	0.33	0.45
OMEGA	0.14*	0.09	-0.20	0.50	0.08	0.10	0.98*	0.60
St. dev.								
SP	1.94***	0.10	1.94***	0.10	2.07***	0.12	2.06***	0.10
IN	0.00	2.27	0.01	1.96	2.33***	0.15	2.29***	0.13
PRO	0.92***	0.09	0.89***	0.09	0.04	0.21	0.05	0.22
OMEGA	0.51***	0.12	0.55***	0.12	0.70***	0.13	0.69***	0.12
St. dev. of EC	2.37***	0.11	2.38***	0.12	2.11***	.08	2.12***	0.07
Log likelihood	-4043.58		-4037.81		-3863.21		-3850.70	
Chi^2 statistic	2591.35		2602.89		2508.25		2533.27	

*** signifies $p < 0.01$; ** signifies $p < 0.05$; * signifies $p < 0.1$

Overall, we can say that raw meat color certainly influences consumer preferences for chicken breast; where yellow tones are preferred and orange hues are rejected. Future research should focus on evaluating consumer preferences for measurable (quantitative data) meat color in order to best utilize resources and technologies for influencing meat color (Mancini and Hunt, 2005) to allocate global (and local) supplies with regional consumer demands to increase welfare gains.

Both labels significantly influenced consumer choices for chicken breast, adding to the literature that sustainability labels (e.g., Grunert et al., 2014; Van Loo et al., 2014) and health claims can influence consumer preferences for animal products; however, health claims play only a secondary role in determining preferences (De Marchi et al., 2016; Hoek et al., 2017). Health claims can be perceived as untrustworthy (Talati et al., 2016) and unconvincing (Hung et al., 2017), which may be one reason for their weakened influence on consumer choices. Overall, consumers use labels to receive information regarding credence attributes even in the face of an abnormal product appearance, suggesting the possibility of 'nudging' consumers towards chicken breast produced with alternative feedstuffs using well-established labels. Sustainability claims and labels appear to be the most promising.

The second scenario of providing consumers with information and labelling on the feed ingredients used in production unsurprisingly increases consumer heterogeneity; such a phenomenon is often observed with the provision of information, e.g., Bronnmann and Hoffmann, 2018; Grebitus et al., 2013a; Risius and Hamm, 2017. More importantly, acceptance for novel feedstuffs increased with the provision of information. On average, informed consumers prefer chicken breast produced with insect meal; mean coefficients are even larger than the uninformed model. Simultaneously, the standard deviation is large and significant. This illustrates that information and an indication of feedstuff changes the intensity of preferences for numerous consumers. Preferences and their intensity diverge, which is consistent with much of the literature on the Western readiness to consume insects directly (Gere et al., 2017; House, 2016; Menozzi et al., 2017; Verbeke, 2015). Onwezen et al. (2019) found Western consumers more open to insects as feed compared to food; therefore, our findings validate the strategy proposed by Marberg et al. (2017) to increase consumer exposure and acceptance of insects by including them in feed.

Overall, information leads to greater consumer acceptance for both alternative feedstuffs. Despite heterogeneous preferences, information was able to lead to consumer acceptance of chicken breast produced with spirulina; mean coefficients are positive, yet not significant, meaning that informed consumers are not willing to pay more than the status quo for chicken breast spirulina labelled with spirulina as a feed ingredient (Figure 2). Whereas, information with insect meal label results in a WTP of 2.54 € pro 500g package; this is not trivial for industry. As spirulina and insect meal are currently more expensive to procure than soybean meal (Chen et al., 2016; Veldkamp and Bosch, 2015), an increased price for the end-product (chicken breast) will be necessary to compensate for higher input costs. In this regard, the incorporation of insect meal appears more feasible from a consumer perspective, as consumers show a willingness to carrying at least some of the additional input costs. Our results show that information can bridge the knowledge gap on raw meat color so that consumers may accept chicken breast produced with spirulina.

a)

b)

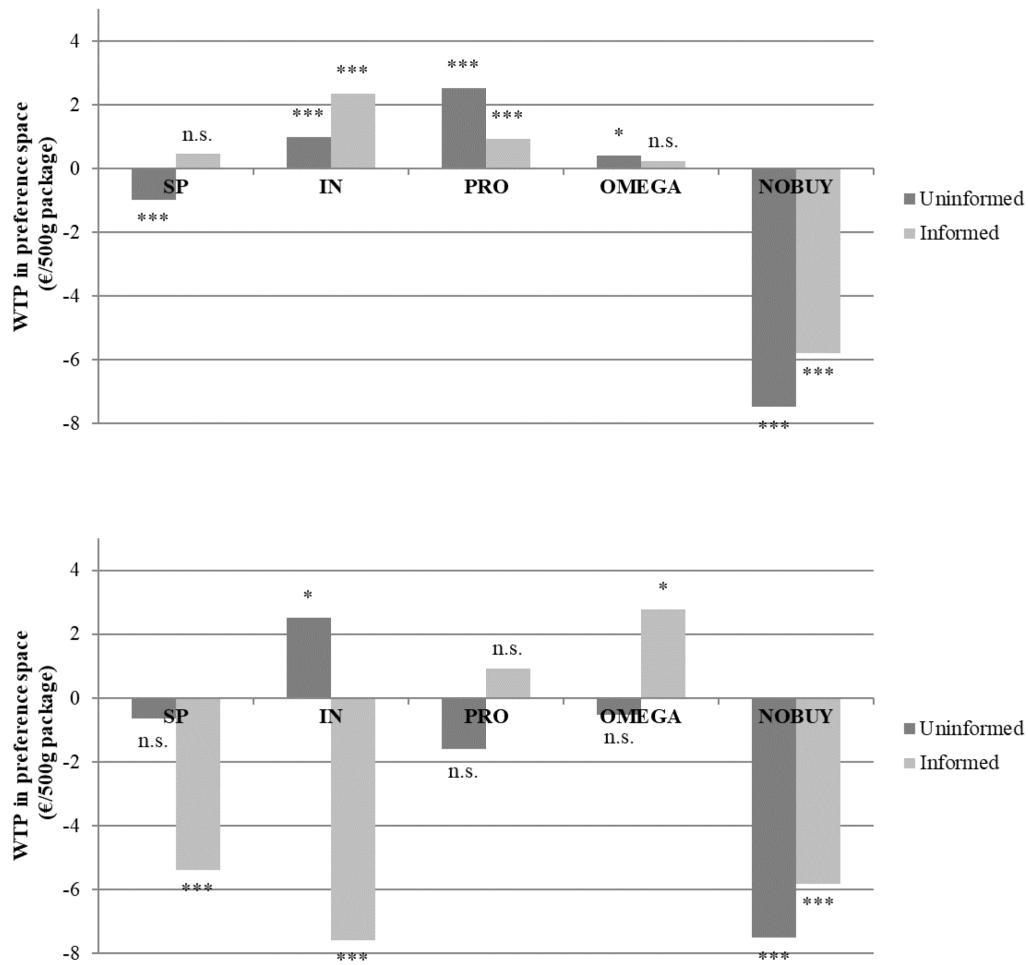

Figure 2: Willingness to pay (WTP) in preference space for attribute variables in standard (a) and NEP interaction (b) models.

Labels only weakly influenced consumer choices in the informed situation, which may have two potential reasons: either search attributes are preferred to credence attributes; or transforming a subjective sustainability claim into a tangible property more strongly influences consumers. Ardeshiri and Rose (2018) conclude that consumers prefer search attributes and may perceive cues regarding specific credence attributes, such as healthiness, from specific search attributes. In addition, an uncomplicated label, such as feedstuff, could be perceived as more transparent and is more understandable to a consumer than a complex general sustainability label, e.g. ProPlanet (Pouta et al., 2010; Samant and Seo, 2016; Venus et al., 2018). Although our study did not focus on replacing or transforming credence attributes; future research should investigate the ability of raw meat color and feedstuff identification (with or without additional information) in replacing sustainability credence labels.

To further understand consumer preference dynamics, we included interaction effects with a psychometric to account for consumer environmental-consciousness (NEP). NEP is a well-established attitudinal scale. Steiner et al. (2017) incorporated NEP scores to identify consumer groups and improve latent class model explanatory power. Similarly, including interaction effects between consumer NEP values and choice variables assists in appropriately allocating preference heterogeneity based on differing degrees of environmental-consciousness. The validity of our model is supported by the positive and highly significant PRO*NEP mean coefficient in for the uninformed consumer sample, showing that environmentally-conscious consumers are drawn to the sustainability label.

Consumer attitudes in combination with belief around product qualities influence intention to act. Therefore, it is important to model consumer attitudes in a situation where product qualities (information and feed indication) are known, so as to appropriately model choice preferences. When taking environmental-consciousness into account, as in the NEP interaction model, consumer preferences change drastically. On average, an environmentally-apathetic consumer rejects chicken breast produced with insect meal; WTP (- 7.59 €) is less than the opt-out option (Figure 2). A similar phenomenon is observed for spirulina as feed. Such reactions likely stems out of disgust as consumers contemplate insects within their food production system (La Barbera et al., 2018; Menozzi et al., 2017); however, we assume this proportion of consumers to be small. This consumer sub-set could be won over to eventually prefer insect-fed chicken breast through tastings and exposure (Deroy et al., 2015; Menozzi et al., 2017), given that insect meal does not degrade organoleptic meat quality in chicken breast (Altmann et al., 2020).

On the other hand, the SP*NEP and IN*NEP mean coefficients are positive and significant. Environmentally-conscious consumers have a high degree of preference towards the alternatively-fed chicken breasts, when they have information allowing them to differentiate the products from one another. This exemplifies the important role that information and feedstuff indication play for niche consumer groups. In our study, FNTS and Wellness scores did not attribute to increased model explanatory power, nor clarity. This could be because neophobia scales do not capture affective factors, such as disgust (La Barbera et al., 2018). Important factors explaining consumer preferences for sustainably atypical products, beyond environmental-consciousness, consist of a complex network of disgust, perceived healthiness, as well as traditionalism/familiarity (Powell et al., 2019). NEP was satisfactory in explaining preferences for chicken breast produced with insect meal. Using feed ingredients as a marketing strategy for poultry products appears feasible, especially to increase welfare gains with environmentally conscious consumers.

Important to evaluate validity, our models (Table 3) produce results consistent with utility theory; namely, all price coefficients are negative and highly significant, indicating that increasing price reduces utility derived by consumers. In addition, the NOBUY (opt-out) coefficient is highly negative and highly significant, signaling that consumers perceive chicken breast as delivering positive utility gains. Using the price coefficient, we further analyzed willingness to pay (WTP) in preference space.

5.0 Policy Implications:

In short, we elicit consumer preferences based on two scenarios: 1) industry introduces alternative feedstuffs without consumers being made aware (uninformed); 2) consumers receive information on feedstuffs and the feed ingredient is declared on the package (informed). Both developments are plausible in the livestock sector, even simultaneously. For example, in Germany, livestock products produced with genetically-modified feedstuffs do not need to be declared (scenario 1); yet, there exist non-GM publically regulated voluntary declaration (scenario 2) schemes. These schemes are continually gaining ground and major meat retailers in Germany have already stipulated to only shelve products bearing "GMO-free" labelling.

Scenario 1 presents losses compared to the status quo, especially concerning spirulina as a feedstuff. The unfamiliar raw meat color is not accepted and requires further explanation in order to make the chicken breast marketable. On the other hand, consumers prefer insect-fed chicken breast based on the resulting appearance, i.e. yellow flesh. However, our investigation of the role of environmental motivation indicates that if it were to come out that insects were being fed as a feed ingredient, this information could be met with extreme backlash from environmentally-apathetic consumers. Depending on numerous factors, e.g. media coverage, such an event could evolve into a food scandal. Especially in the social media age, controversial agro-food topics generate a lot of attention. Potentially, this loss of face would result in decreasing consumer acceptance of poultry products in the short-term (Rieger et al., 2016) as well as a damaged reputation and loss of consumer trust. Therefore, in response to consumer demands for enhanced standards and declarations (Agnoli et al., 2016), the agro-food industry should be proactive instead of reactive (Busch et al., 2018).

For the most efficient integration of alternative feedstuffs, a declaration of feed ingredients could be advisable (scenario 2), contingent on information being a precursor to clarify observable changes in product quality, such as meat color. In this case, industry would forgo opportunity costs associated with rejection based on raw meat color, or a loss in consumer trust and reputation. Moreover, chicken breast produced with insect meal could be differentially priced for environmentally-conscious consumers. Currently to be viable, an increased price would be necessary to compensate for increased input costs associated with insect meal (Veldkamp and Bosch, 2015). Similar to the niching of production systems for eggs, the declaration of feed ingredients in meat-type chicken production can allow consumers to employ their purchasing-power towards products appealing to their attitudinal values.

Industry would surely profit from voluntarily labelling feed ingredients; yet, a mandatory declaration policy would be more beneficial to the public at large. Zilberman et al. (2018) recommend mandatory declaration schemes, compared to no or voluntary declarations, as the welfare gains are larger. Mandatory labelling sends a stronger signal to consumers (Venus and Wesseler, 2015) so that not only the asymmetrical information gap between producers and consumers can be bridged, but consumers would feel secure in the provision of the improved standard of increasing meat product information (Agnoli et al., 2016). The infrastructure necessary for a mandatory declaration on animal products already exists in Germany. Unprocessed meat products have a stamp indicating slaughterhouse as well as country of origin, which could be augmented to include farm-based production factors, especially in highly integrated production chains. Such declaration infrastructure is already applied for the declaration of raw eggs in Europe.

The current stamp on meat products is subtle and customarily located on the back of a package and serves primarily for traceability; secondarily, well-informed consumers may use it to gain information on product origin (Bundesministerium für Ernährung und Landwirtschaft (BMEL), 2019). This subtle means of indicating product attributes, such as feed ingredient, is likely a feasible compromise to appeal towards higher willingness-to-pay for insect meal from both environmentally-conscious consumers, who are likely to be more well-informed and pay attention to such a label, and those basing preferences solely on product appearance (and are likely to ignore such a stamp). Objective labelling of production system factors has proven successful in Europe regarding egg production leading industry to offer highly differentiated products (cage-free aviary, free-range, organic) and prices; something that has been highly debated in USA despite clear consumer preferences for a mandatory labelling system (Ochs et al., 2019; Tonsor and Wolf, 2011). Our research illustrates an opportunity to translate the learnings of the European laying-hen sector into the meat sector.

Finally, there remains the potential for poultry producers to institute bio-marking or product quality schemes, where consumers are not solely reliant on credence labels; rather influencing meat color through feedstuffs could transmit aspects of a product's sustainability claims. Consumers trained to equate color with sustainable attributes, such as feed ingredients, can then rely on intrinsic meat color as opposed to trusting an extrinsic label; analogous to consumers using search attributes, such as marbling, to receive information regarding specific health aspects (Ardeshiri and Rose, 2018). This is relevant given that when over stimulated by labels, consumers may react negatively towards labels (Moon et al., 2017); therefore, converting credence quality attributes into search attributes is one way to increase credibility in the eyes of the consumers (Karstens and Belz, 2006). This is one important strategy to strengthen consumer trust in normative product attributes, without off-putting consumer quality expectations.

6.0 Limitations and Future Research:

Our study is the first of its kind to investigate consumer acceptance for alternative feeds. Although we were able to answer our research questions that abnormal appearance (meat color) detrimentally effects consumer acceptance and that insect meal will likely be accepted, especially by environmentally-conscious consumers; our study has limitations and prompted further questions. The threshold of what constitutes an abnormal color for chicken breast still remains ambiguous, and should be investigated in further detail in order to ascertain if spirulina would be accepted as a feed ingredient, when fed at lower doses, i.e. resulting meat has reduced orange hues. Furthermore, due to our experimental design, we were not able to capture the possible gains (or losses) from a feed ingredient label alone, without the prior provision of information. Pertinent to achieving an efficient integration of alternative feeds into livestock production chains, future research should also focus on investigating the effectiveness of exposure and taste-testings to win over potentially disgusted consumers. Finally, latent class analysis should be employed to identify niche markets and their consumers (e.g. organic, 'foodies') in order to inform the markets likely to act as a spring-board for further innovation and exposure of livestock sustainability labelling.

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Appendix

Table A1: Samples description based on total and split sample(s) compared to German census data

Demographic Attributes	**Total Sample**	**Uninformed**	**Informed**	**German Census Data[1]**
Age				
18 – 24	3.63%	3.33%	3.93%	7.7%
25 – 39	25.05%	24.81%	25.28%	18.9%
40 – 59	39.20%	39.81%	38.58%	29.8%
60 – 64	12.66%	11.67%	13.67%	6.3%
65+	19.46%	20.37%	18.54%	21.1%
Female	50.47%	49.07%	51.87%	50.7%
Highest Attained Level of Education				
no qualification	0.56%	0.56%	0.56%	7.6%
Intermediate qualification	49.72%	52.03%	47.38%	58.9%[2]
University qualification	20.39%	19.26%	21.54%	10.2%[3]
University degree	29.33%	28.15%	30.52%	23.3%
Household Income per Month (Gross)				
Sample Mean	2913 €[4]	2880 €[4]	2946 €[4]	4196 €
< 1000 €	8.85%	9.44%	8.24%	N/A
1000 – 2500 €	29.89%	28.70%	31.09%	N/A
2501 – 4000 €	31.66%	33.15%	30.15%	N/A
4001 – 5500 €	13.31%	13.70%	12.92%	N/A
5500+ €	8.10%	6.48%	9.74%	N/A
Not stated	8.19%	8.52%	7.87%	N/A
Attitude Scales (5pt hedonic scale)				
FTNS	3.32 ±0.55	3.35±0.56	3.29±0.54	N/A
NEP	3.77 ±0.54	3.80±0.53	3.73±0.55	N/A
WELLNESS	3.26 ±0.47	3.29±0.47	3.23±0.46	N/A
self-identified as mainly responsible for grocery shopping[5]	77.47%	73.89%	81.09%	N/A
Self-identified percentage of participation in the household grocery shopping				
< 25%	2.23%	3.15%	1.31%	N/A
25 – 50%	16.48%	17.78%	15.17%	N/A
51 – 75%	17.78%	16.48%	19.10%	N/A
76-99%	24.95%	23.70%	26.22%	N/A
100%	38.55%	38.89%	38.20%	N/A
Frequency of purchasing chicken breast				
Daily	0.65%	0.37%	0.94%	N/A
Multiple times per week	8.01%	7.41%	8.61%	N/A
Once per week	30.63%	31.30%	29.96%	N/A
Once every few weeks	33.99%	33.33%	34.64%	N/A
Once per month	10.71%	12.04%	9.36%	N/A
< once per month	16.01%	15.56%	16.48%	N/A

[1]As reported from the German Statistical Office in *Statistisches Jahrbuch 2017* (Statistisches Bundesamt, 2017)
[2]pertaining to *Hauptschul- and Realschulabschlüsse*
[3]As no statistic is available for the highest achieved level of education; this statistic was derived from subtracting the proportion that successfully hold university degrees from the proportion of population who attained a university qualification.
[4]Estimated based on mid-values of income brackets; exception 5500+€ bracket, where a value of 5501€ was used.
[5]remainder of respondents identified as participants in grocery shopping, but not THE responsible person for grocery shopping

Author Contributions: The over-arching project was conceived by Prof. D. Mörlein and his initial contribution and idea led to this experiment. Survey conception was completed by Ms. B. Altmann with the support of Prof. S. Anders. Dr. A. Risius calculated the discrete choice experimental design and provided input on the online survey structure and samples size. Ms. B. Altmann conducted and monitored data collected. Data were primarily analysed by Ms. B. Altmann, with the guidance from Prof. S. Anders and Dr. A. Risius. The manuscript was written by Ms. B. Altmann and revised by Prof. S. Anders (Chapters 1 & 2) as well as Dr. Risius (Chapter 3).

General Discussion

Scientific contribution

Using the foundation of meat science literature (*Fundamental Aspects of Meat Quality*), the research presented in this dissertation addresses the practical concerns of possible trade-offs in physicochemical as well as organoleptic meat quality should production move towards including alternative protein sources in swine and poultry diets. The project chose to investigate spirulina and black soldier fly larval meal because information, although still scarce, was available regarding protein source nutritional composition, and possible impacts on animal health. At the inception of this dissertation (2015) there remained a limited understanding of the effects these two protein sources could have on poultry and pork quality. Given that livestock and poultry are housed first and foremost as a means of meat (or egg) production, the impact of feed on meat quality therefore remains a critical aspect to investigate prior to recommending sector-wide use of alternative protein sources.

In the mid-1990s to early 2000s, it became well-understood that feeding spirulina to poultry has implications for meat colour (Toyomizu, Sato, Taroda, Kato, & Akiba, 2001; Venkataraman, Somasekaran, & Becker, 1994). In 2013, Holman et al. (2013) published a review on spirulina as a feed supplement, where it is explicitly stated that research had up until that point primarily focused on incorporating spirulina into poultry diets. Furthermore, research had been focused on spirulina as an animal feed supplement, not as a primary animal feed ingredient. Those studies also focused on important animal health and nutrition aspects (Holman & Malau-Aduli, 2013). The potential changes in meat quality are identified throughout this dissertation so as to best incorporate spirulina as a feed ingredient without losing producer, processor, and consumer trust.

Black soldier fly as an animal protein source has been broadly investigated and advocated by Newton et al. since the late 1970s. However until recently, research has been mainly limited to the working groups of Newton and Sheppard, which focused on developing insect-rearing and animal husbandry systems (Newton, Sheppard, Watson, Burtle, & Dove, 2005), as well as investigating the feed ingredient's ability to replace fish meal in fish as well as poultry and swine diets (Newton, Booram, Barker, & Hale, 1977; Newton, Sheppard, & Burtle, 2008). These studies focused on animal nutrition aspects; although St-Hilaire et al. (2007) also investigated fatty acid composition and Sealey et al.(2011) the sensory profile of rainbow trout fillets. As replacing fish meal was the main driving force for this collaborating group of researchers, the main species of production was fish in aquaculture systems. Later on, a stronger trend developed to investigate and advocate for insects as an alternative protein source in terrestrial animal feed (Govorushko, 2019; Józefiak et al., 2016; Makkar, Tran, Heuzé, & Ankers, 2014); however, the effect of insect meals, especially black soldier fly larval meal, on product quality are often not included in studies.

The task of Paper 1 was to preliminarily investigate the effect of both feeds on the broiler meat quality. The fundamental physicochemical meat quality aspects were monitored and compared across feed groups; status-quo broilers fed a soy-based diet accompanied the trial as a control group. Sensory profiling was also conducted using a limited number of animals. This study included industrial factors, such as industrial packaging and storage conditions; however due to limited supply of animals packaging and storage time remained confounded factors in the design. In addition, the chance for type I or type II errors were rather high, given the very small sample size. Overall, the pilot study assisted in rejecting the null hypothesis that neither alternative feed effects meat quality, as well as the findings informed the planning of future studies with larger samples sizes. This research was highly explorative and indicated the first implications that feeding spirulina and black soldier fly larval meal can have in effecting broiler meat quality. The lack of such information within the scientific community is apparent on the number of citations for such a niched pilot project. Since its publication in 2018, the paper has been cited a total of 13 times as of June 2020.

Paper 2 follows up on the pilot study included in Paper 1. The aim of Paper 2 was to replicate the findings of Paper 1 with a larger sample size and non-confounded variables (decouple time and packaging). It is important to repeat experiments as part of the scientific process in order to verify results. Furthermore, the insights into packaging type and time can provide useful insights for industry on how to best adjust their packaging and distribution lines should alternatively-fed broiler products go to market. Paper 2 also includes the fatty acid profile of intramuscular (thigh) fat. This parameter is able to better inform lipid oxidation results, as well as provide information on the overall nutritive profile of alternatively-fed products. Paper 2 does have one drawback compared to Paper 1; sensory analysis was not conducted with packaged and stored samples, as would be typical in an industrial setting. Paper 1 reported an effect of storage on the sensory profile of broiler meat produced with black soldier fly larval meal. Such an effect could neither be verified nor rejected, yet would be relevant from an experience eating quality perspective (i.e. consumer), which is important for repeated purchasing behaviour. The impact of Paper 2 is not yet known in the broad scientific community, as it was only recently published. Nonetheless, the results in Paper 2 were able to generally verify some findings, such as spirulina positively altering product flavour and colour. Due to its improved experimental design and larger sample, the findings in Paper 2 should be interpreted as more reliable compared to the first findings in Paper 1. The rarity of studies including two alternative feed ingredients in one trial, as well as the focus on comprehensively investigating meat quality aspects sets Paper 2 (as well as Paper 1) apart from previous studies on spirulina or black soldier fly larval meal as alternative protein feeds.

Paper 3 summarizes two trial replicates in order to determine the effect of spirulina and black soldier fly larvae, as protein feed sources, on pork quality. Overall meat quality was interpreted based on the investigation of the *longissimus thoracis et lumborum* (LTL) muscles. Due to the overall size difference between a swine and a broiler, and therefore availability of sample material, all physicochemical parameters as well as sensory analysis were investigated on every animal. Something that was not possible in Paper 1 nor Paper 2, where the design allocated animals to the investigation of specific parameters. Experimental diets were not identical between the two swine trials included in Paper 3; however, the finishing diets (fed at >75kg live weight) were identical and therefore the trials were analysed together, while including "replicate" as a random factor in the mixed models. Subcutaneous fat from the neck was used to analyse fatty acid composition. To our knowledge, this is the first study to both investigate the meat quality, and especially fat quality, of swine fed spirulina or black soldier fly larval meal. As with the Paper 1 and Paper 2, Paper 3 is also innovative in that both alternative protein feeds were included in the same experimental design. Overall, due to the fact that swine finishing diets tend to be rather low in protein, compared to broiler diets, Paper 3 concludes that neither spirulina nor black soldier fly larval meal play an important role in determining meat quality. One exception, black soldier fly larval meal does have a significant impact on subcutaneous fatty acid composition, of which the implications and potential should be further investigated. To date (June 2020), Paper 3 has been cited a total of 5 times.

The literature findings as well as the replicated results in Paper 1 and Paper 2 regarding meat colour presented an innovative opportunity for investigating consumer preference for poultry meat colour as well as chicken breast labelled as alternatively-fed. Within Paper 4, a discrete choice experiment embedded within a consumer survey to provides insights into consumers' willingness to pay for alternatively-fed chicken breast. The visually altered chicken breast arises from the spirulina-fed broiler that has a more intense colour (orange opposed to white-pink); uninformed about the origins of the chicken breast, half of the consumers were asked to choose between either chicken breast produced with a status-quo (soybean), spirulina-based, or black soldier fly larval meal (insect)-based diet. The other half received information about the differing feeds used in production and then the respecting chicken breasts were labelled "spirulina-fed", "insect-fed", or "soy-fed". Consumers without information reject spirulina-fed chicken breast on the basis of appearance; consumers prefer the appearance of insect-fed chicken breast. When informed and products are labelled, consumers accept (but do not prefer) spirulina-fed chicken breast, the willingness to pay for insect-fed products increases. To date outside of the GMO vs. non-GMO debate, there only exists one other choice analysis experiment, which investigates consumer preferences toward feedstuffs used in the production of animal products (Profeta & Hamm, 2019). Profeta and Hamm (2019) focused on the geographical origins (local) of feeds, as opposed to specific feed ingredients. The results of Paper 4 are widely applicable, as they report implications for policy, marketing, as well as consumer acceptance of alternatively-fed chicken breast.

First, the results of Paper 4 can be applied to better understand the importance of raw meat colour at the point of purchase, as well as "how red is too red" for white meat products. This is specifically relevant as current (highly oxygenated modified atmosphere) packaging is applied solely to increase the visible red colour of raw meat products. To date, few studies focus on determining consumer preferences for raw (pork or beef) meat colour (Grebitus, Jensen, Roosen, & Sebranek, 2013; Lusk, Tonsor, Schroeder, & Hayes, 2018; Ngapo, Martin, & Dransfield, 2007). No discrete choice experiments are known to take exist, where poultry meat colour is included as an individual attribute; although focus group studies regarding consumer preferences for chicken breast quality do exist (Kennedy, Stewart-Knox, Mitchell, & Thurnham, 2005).

Secondly, Paper 4 informs consumer preferences towards feeds as a production system input. There already exist numerous studies investigating consumer preferences for various livestock husbandry systems or other related sustainability labels (Pouta, Heikkilä, Forsman-Hugg, Isoniemi, & Mäkelä, 2010; Risius & Hamm, 2017; Samant & Seo, 2016; Van Loo, Caputo, Nayga, & Verbeke, 2014). However, feeds as a factor where consumers may have preferences has remained relatively undiscussed, with the exceptions outlined above. Numerous papers on insects as food and feed often report that insects may likely be more widely accepted as an animal feed source (Józefiak et al., 2016; Makkar et al., 2014; Onwezen, van den Puttelaar, Verain, & Veldkamp, 2019); Paper 4 is the first consumer study to my knowledge to confirm these conjectures using a real product. Unfortunately, Paper 4 was not yet published in a scientific journal at the time of the publication of this dissertation; however, there is no doubt that with its innovative application of a discrete choice experiment together with a socially-relevant topic that Paper 4 will be well-received by the scientific community.

All four papers contribute to understanding the potential of spirulina and black soldier fly larvae as protein sources along the poultry and pork value chains. It can be concluded from a physicochemical standpoint that both alternatives produce meat with comparable or improved quality; however, processors' and consumers' preferences as well as requirements need to be taken into account in order for alternatively-fed meat to meet its full potential. At the completion of this dissertation (2019), insects as an alternative protein source continues to be a dynamic field of research driven by the high demand for protein and sustainability concerns related to arable farming, world-wide.

Project limitations

Due to its multi-disciplinary structure, the over-arching project was able to identify limitations to the overall success of implementing the results of this dissertation; nevertheless, project boundaries still exist. The following sections outline some of the possible lock-ins and/or constraints that are limiting the likelihood of spirulina and black soldier fly larvae becoming commonplace in broiler chicken and pork production. In addition, some missing information, such as economic viability, are highlighted.

Sustainability

Sustainability is most often defined using the *Report of the World Commission on Environment and Development: Our Common Future*, often referred to as the Brundtland Report (1987), definition for sustainable development (United Nations, 1987). This definition states that humanity should make decisions that ensure present needs can be met without endangering the ability of future generations to meet their own needs. Three aspects are crucial for assessing sustainability: the environment, society, and economies. Due to its breadth, sustainability is a difficult construct to objectively measure, especially given that it has a future component which can only be estimated or speculated. Nonetheless, numerous methods have been proposed to estimate the sustainability of a process or policy.

In food production, a life cycle assessment (LCA) is commonly used to estimate the sustainability of one product from conception to grave compared to other options. The method has been around nearly as long as the concept of sustainability (Klöpffer, 2002). In an LCA, all system inputs and outputs are converted into a functional unit in order to objectively compare unique products; ISO standards are recommended for conducting a LCA (Klöpffer, 2002). Although there are many critique points, such as the non-transparent functional unit conversion and focus on environmental compared to social (usually only health consequences) and economic aspects, the method is widely accepted as the basis for estimating sustainability in scientific works.

Therefore, as a part of the over-arching project, LCAs were conducted to determine the sustainability of substituting soybean meal with spirulina or black soldier fly larval meal, compared to no substitution. Results show that the two alternative protein sources included in this dissertation are in fact less sustainable than the status quo – soybean meal imports (Smetana, Palanisamy, Mathys, & Heinz, 2016; Smetana, Sandmann, Rohn, Pleissner, & Heinz, 2017; Ye et al., 2018). This is explained by multiple factors, which differ between the two alternative protein sources.

The production of spirulina requires large amounts of energy when produced in temperate regions (Smetana et al., 2017). Spirulina requires a liquid, typically alkaline, medium high in nutrients such as nitrogen, phosphorus and potassium; in order to achieve effective yields, the medium should be warmed to around 30°C (Chen et al., 2016). Spirulina is traditionally produced in bioreactors, which are difficult to upscale, or open race-way ponds (in warming climates), which are water inefficient (Chen et al., 2016). In other words, spirulina requires a number of natural resource inputs that drastically affect the sustainability of its production.

Recent efforts have investigated the possibilities of including waste products as nutrient sources for spirulina production. Studies using biogas effluent (Hultberg, Lind, Birgersson, & Asp, 2017), swine wastewater (Olguín, Galicia, Mercado, & Pérez, 2003), and other agro-industrial waste sources (Markou & Georgakakis, 2011) as nutrient sources have proved successful. This is a mutual solution, as spirulina also has the capacity to treat waste sources by reducing organic pollutants (Markou & Georgakakis, 2011), particularly nitrogen and phosphorus.

Other microalgae cultivars could prove more advantageous for protein production than spirulina (Smetana et al., 2017). Taelman, De Meester, Van Dijk, da Silva, & Dewulf (2015) studied the production sustainability of an algae mix of *Scenedesmus spp.* and *Chlorella spp.* production in the Netherlands using an innovative pilot bio-refinery approach, where ponds were warmed using surplus heat from a biogas reactor. However, soybean meal remained a more sustainable option to the algal mix. Preparing nutrient and algae starters proved the most inefficient part of the production process; product drying also required large amounts of energy. Nonetheless, meeting energy requirements for drying with renewable sources (e.g., wind, solar, etc.) and increasing microalgal productivity could be enough to match the sustainability of soybean meal.

In conclusion, research initiatives should turn to more productive microalgae cultivars, such as *Chlorella spp.*, and focus on increasing yields while using waste product inputs in scalable, low-water input refineries. Additionally, microalgae production should take place in climatic regions favourable to growth to reduce energy inputs and increase sustainability. A tremendous amount of innovation and research remain necessary to make microalgae viable as a sustainable alternative to soybean meal.

On the other hand, black soldier fly larvae are one of the easiest insects to rear (Józefiak et al., 2016; Makkar et al., 2014) and have a higher feed efficiency rate compared to other insects produced for protein, such as crickets (Oonincx, van Broekhoven, van Huis, & van Loon, 2015). However, in Europe their sustainability potential is highly impaired by legislation. Since they are animals, insects must be fattened on approved animal feedstuffs (Lähteenmäki-Uutela, 2017). This puts their production in direct competition with swine and poultry; effectively, rearing black soldier fly larvae for animal feed is just adding an additional heterotrophic level.

In the name of sustainability, larvae should be reared on substrates, such as municipal organic wastes (Diener, Studt Solano, Roa Gutiérrez, Zurbrügg, & Tockner, 2011) vegetable waste (Spranghers et al., 2017), animal waste (Sheppard, Larry, Thompson, & Savage, 1994) and even faecal waste (Lalander et al., 2013), which have proven to result in adequate larvae yields. In addition, black soldier fly larvae prefer a warm ambient temperature of ~ 30°C and a high relative humidity (70%) (Oonincx et al., 2015); therefore, similar to spirulina, their production efficiency greatly depends on climatic conditions. One black soldier fly larvae producer in Germany (Hermetia Baruth GmbH) is able to reduce its energy requirements by using surplus heat produced from a neighbouring biogas reactor. Utilizing waste side-streams as substrates and renewable energy is necessary to achieve a more sustainable production, which could potentially compare to that of plant-based proteins (Smetana, Schmitt, & Mathys, 2019).

Legalities, health, and safety

As a part of the food system, feedstuffs are highly regulated in the EU in order to ensure food for consumption remains healthy and safe. Spirulina and black soldier fly larvae present unique challenges to ensuring that feedstuffs remain healthy for livestock and poultry, and therefore the end consumer.

Recreational lake-users will immediately associate microalgae with dangerous toxins (and missing out of a nice day at the beach). Although spirulina does not produce toxins like other cyanobacteria (blue-green algae) that lake-goers are wary of, in open race-way ponds the possibility exists that another microalgae species could contaminate spirulina production with anatoxins or microcystines. Water contaminated with microalgae that produce anatoxins (neurotoxin) has been known kill pets and livestock (Health Canada, 2012). Microcystines (heptatotoxins) can also be produced by specific microalgae, which affect liver function (Health Canada, 2012). Open race-way ponds also present the risk of microorganism contamination (van der Spiegel, Noordam, & van der Fels-Klerx, 2013), and heavy metal contamination also remains a safety concern (van der Spiegel et al., 2013). Closed-system production and bioreactors should be implemented wherever possible to reduce the chance of contamination. In addition, spirulina should be proved free of toxins prior to being included as a feedstuff; under EU law it must be proven below legislated thresholds for heavy metals and other contaminants according to Directive 2002/32 (European Parliament, 2002). Finally, unlike insect meal, spirulina is approved as an animal feedstuff in the EU.

Currently, numerous legislative barriers in the EU impair an effective and efficient incorporation of insect meal as an animal protein feed. Currently, insect meal is only approved for pet food and use in aquaculture production (Lähteenmäki-Uutela, 2017); although it is anticipated that an approval for poultry production will be granted in the near future. Therefore, despite the positive results presented in this dissertation, black soldier fly larval meal is not allowed to be incorporated into swine and poultry diets in the EU. Special permission was granted by the veterinary bureau ("*Veterinäramt*") of Lower-Saxony for conducting the sensory analysis in this dissertation. Nonetheless, this research may be of interest for poultry production elsewhere, such as in Canada where an authorization was recently passed to allow poultry production using industrially produced black soldier fly larval meal as a feedstuff, if the substrate guarantees a "safe and nutritious" feed (Lähteenmäki-Uutela, 2017).

Although not permitted as a terrestrial animal feed, the rearing of insects is not prohibited in the EU. As briefly mentioned above, the production of insects is regulated so that animal feedstuffs are required as the fattening substrate. This too may change; currently, the International Platform of Insects for Food and Feed (IPIFF) is lobbying for the permission to use vegetable substrates (Lähteenmäki-Uutela, 2017). Substrate is an extremely sensitive topic, because insect meal must remain below the thresholds for heavy metals and other contaminants as set out for other animal feedstuffs Directive 2002/32 (European Parliament, 2002). Black soldier fly larvae have been proposed for decades as a means to (re-)cycle waste products into high quality feed products (Newton et al., 2008). However, numerous regulating bodies are skeptical about allowing grey or black waste-streams as a substrate due to the possibility of bio-accumulation and other contamination risks. Larvae can survive in heavy metal contaminated substrates; although productivity decreases (Diener et al., 2011). In addition, bioaccumulation of heavy metals has been confirmed in black soldier fly larvae reared on substrates of varying contamination concentrations (van der Fels-Klerx, Camenzuli, Van Der Lee, & Oonincx, 2016). These findings highly restrict the likelihood of authorization for black soldier fly larvae production to utilize grey or black waste streams, especially with the intention to use the larvae as a protein source.

Lastly, it should be mentioned that black soldier fly larvae present a low risk of becoming an invasive species as they considered a non-pest species in Europe (van Huis & Oonincx, 2017). This may not be the case for other insects, such as grasshopper and locust species (van Huis & Oonincx, 2017).

Economic viability

Unfortunately, although the research included in this dissertation did investigate consumer preferences and willingness to pay, the break-even price necessary for economic viability of these products has not been investigated. Even with large-scale production in China, spirulina production costs remain high, making it only viable as a nutritional supplement as it cannot compete with soybean meal prices (Chen et al., 2016). Regarding black soldier fly larval meal, Sánchez-Muros, Barroso, & Manzano-Agugliaro (2014) conclude that animal performance gains derived from insects as feed could compensate for the additional input costs. Production costs may not decrease drastically with large scale well-established production facilities, but economic viability will likely only increase for both of these alternative protein sources with the projected increase in the demand for protein feed (FAO, 2015). Nonetheless, economic viability is likely an important lock-in hindering the transition towards the two alternative protein sources becoming commonplace animal feed.

Future research

The overall feasibility of spirulina and black soldier fly larval meal, and therefore the likelihood of a successful system transformation hinges on product safety and economic viability. Therefore, research should focus its efforts on theses fronts. First, production cost analysis should be conducted for both alternative protein feeds, so that these calculations can be included in cost analysis for the resulting meat products. Subsequently, consumers should be presented with realistic product prices in further consumer preference studies, instead of using current prices for the status-quo chicken breast product.

In regards to spirulina as a protein feed, feeding trials with incremental inclusion rates will assist in investigating 1) the threshold of inclusion where colour differences are not noticeable to the naked eye, and 2) which inclusion rate and resulting colour (if any) do consumers prefer. Studies on consumer preference for meat colour should be conducted cross-culturally for all meat products, especially given that Germany is a poultry and pork exporter.

Extensive research and legislative work is required to formalize the incorporation of insect meal in European animal production. Determining thresholds of substrate contaminants based on reared insect species will require much effort. Identifying biomarkers in meat may also be necessary if insect meal remains blacklisted in the EU while other meat producing countries increase incorporation of insect meals and export to EU markets.

Based on the results of this dissertation, additional studies should focus on establishing the organoleptic attributes of black soldier fly larvae reared on different substrates. Determining the effect of substrate on organoleptic properties in the larvae can help to illuminate the potential for variation in organoleptic properties of meat products produced using larval meal originating from different substrates. Such research is the basis for formalizing sensory profiling of meat products.

Finally, as the over-arching project was based on multiple assumptions around European normative values about the use of soybean meal that have not been academically validated, this presents an extremely relevant and interesting opportunity for future work. Underlying consumer motivations for preferring ‘local feed’ need to be investigated; it is unknown whether this result is a soybean aversion, a GMO aversion, a soybean import aversion due to food security or sustainability concerns, or a preference for domestic origin. In addition, perceptions and consumer latent values regarding soybean meal could be investigated. A think-aloud protocol or focus groups encompassing a large sample of diverse participants across multiple European countries may suffice.

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