# Autochthonous breeds and alternative feeding strategies in poultry production: a multidisciplinary approach towards sustainability and animal welfare

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**Summary**

## Abbreviations

Bianca di Saluzzo: BS

Bionda Piemontese: BP

Black soldier fly: BSF

Black soldier fly larvae: BSFL

BPM: Bionda Piemontese male/males

BPF: Bionda Piemontese female/females

BSM: Bianca di Saluzzo male/males

BSF: Bianca di Saluzzo female/females

a*: redness

b*: yellowness

L*: lightness

SFA: saturated fatty acids

MUFA: monounsaturated fatty acids

PUFA: polyunsaturated fatty acids

pHu: ultimate pH

## 1. Poultry production: state of the art and expectations for the future

Globally, poultry meat represents the main meat consumption exceeding both pig and beef meat request (FAOSTAT, 2020b) and this trend is projected to increase in the next decade, providing the 41% of all the meat protein source (OECD-FAO Agricultural Outlook 2021-2030, 2021) (Figure 1). Nowadays, 23 billion of domestic chickens can be estimated to exist (Qualman et al., 2018). The reason of preference of poultry meat is attributable to the good poultry feed conversion ratios, absence of cultural barriers, brief rearing cycles, and its plasticity in terms of rapid genetic ameliorations, animal health and rearing practices on the basis of the market requirements (OECD-FAO Agricultural Outlook 2021-2030, 2021). Nonetheless, progress in industrialization of poultry production requires excellent and expert management technique and even if the supply chain has been improved in efficiency and safety, the large and medium scale are favored to the detriment of niche production and small farms (FAO, 2023). Particularly, the divergence between this production scales (large/medium and small) regards the inclusion of the first type (large/medium) in integrated value chains, while the extensive systems (small, which represent the main support to local markets) isolated and far from such integrated chains and thus disadvantaged. Moreover, small-scale production still maintains a crucial role in poultry sector of developing countries and rural areas, a reality characterized by widespread misery and scarce or limited livelihood. Unsurprisingly, 80% of poultry production in developing countries derives from rural farms. (FAO, 2023). Considering the world meat and eggs production, the United States represent the greatest contributors in meat products (17%), succeeded by China and Brazil, while the major egg producers are China (38%), United States (7%), and India (7%) (FAO, 2023). Furthermore, considering the increasing in their demand, the meat and egg production raised from 9 to 133 and 15 to 93 million tonnes between 1961 and 2000, respectively (FAO, 2023). Among poultry, chicken meat represents the most consumed meat, accounting for about 21 million metric tons of meat produced in the United States and 11 million metric tons in Europe in 2021-2022 (Statista, 2023). The population growth is the primary factor affecting the consumption of meat together with the economic growth of the countries, being both relevant drivers in consumers' choices. Thus, an increase in meat consumption equal to 30% is expected in Africa, 18% in the Asia and Pacific regions, and 12% in the Latin American region, while this projection is of 9% in North America and 0.4% in Europe in 2030. Similarly, the pro capita meat consumption will be lower in high-income countries while the opposite is foreseen for the countries with a low income. This prediction is hinged on other drivers, such as animal welfare, environmental, ethic and health aspects (OECD-FAO Agricultural Outlook 2021-2030, 2021).

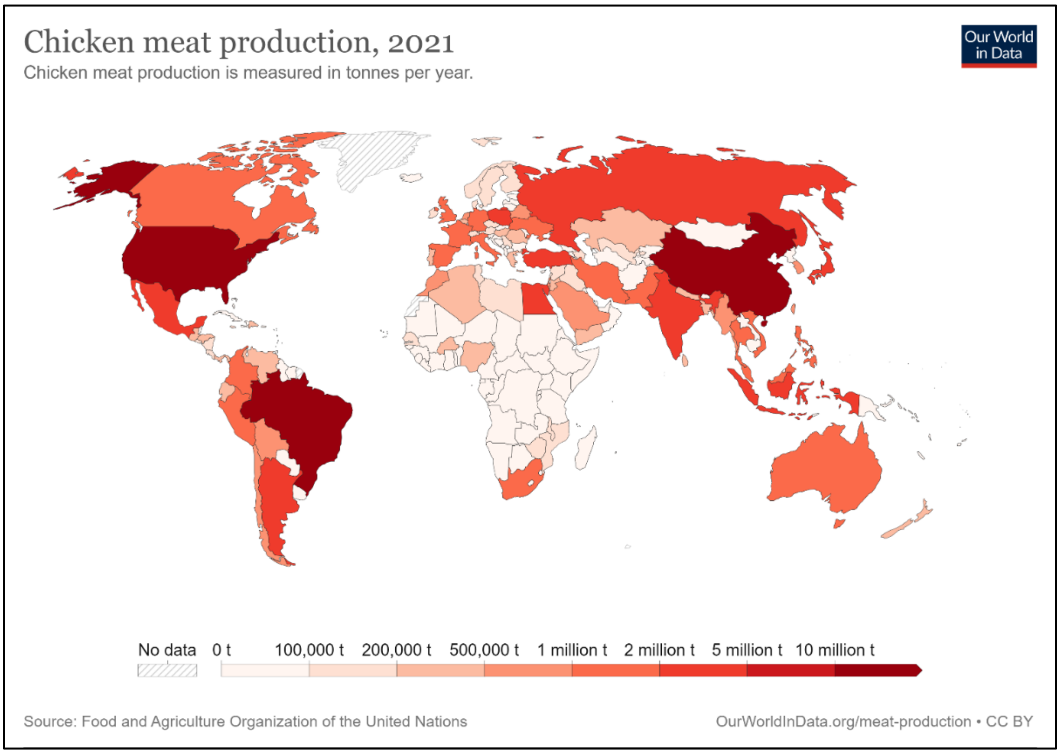



Figure 1. Chicken meat production in 2021 (source: Food and Agriculture Organization of the United Nations).

The poultry product purchase process depended on various drivers, being this system prompted by several factors. Nonetheless, the economic affordability and the preference of breast filets over wings, thighs, and whole animals are still the greatest factors of influence in consumers' purchases, despite the ideal chicken profile has been characterized by good rearing practices, appropriate remuneration of the farmers, production transparency, and origin of the products close by the geographical location of the consumer (Escobedo Del Bosque et al., 2021). Furthermore, the products labelling covers a relevant role in the consumers' perception of the products and their acceptance, especially in terms of animal welfare, by dint of its potential as a guaranteed product grade. Nevertheless, the labelling could represent a confounding element where not properly designed and scant of explanatory insights (Pinto Da Rosa et al., 2021), highlighting the needs for clearer labelling system which could improve products acceptance and their demand on the market.

## 2. Sustainability of poultry production: the challenge of this decade

Undeniably, poultry production satisfies higher sustainability standards than other livestock rearing realities. However, its contribution in terms of global warming, eutrophication and acidification must be recognized. A study conducted in the UK poultry sector revealed that the global warming can be attributed for the 10% to the feed production process and transport, while manure management is responsible of about 40-60% to their eutrophication potential and acidification potential, respectively (Leinonen and Kyriazakis, 2016). In the main poultry producer countries, as the United States, laying hens and broiler chickens broadly contribute to atmospheric pollution due to their excreta nitrogen content (mainly in terms of ammonia emissions) (Bist et al., 2023). Various approaches can be adopted to reduce the environmental impact, which consider adaptation through genetic selection, efficiency in animal and excreta management, and changes in feeding strategies (Leinonen and Kyriazakis, 2016). However, livestock sustainability is made surely by several components such as animal welfare, economics, environmental impact and social justice (Fernyhough et al., 2020).

Interventions on the modulation and change of feed ingredients, especially reducing the soybean present in the diet, is one of the most efficient strategies to increase the sustainability of poultry production (Etemadian et al., 2021). The popularity and success of soybean can be reconducted to the major efficiency of this cultivation than the other crops. Considering the land-use efficiency, 343 kg of cattle meat, 600 kg of pork, or 1200 kg of poultry can be produced starting from a soy yield of 3000 kg per hectare (Garrett and Rausch, 2016), while only 250 kg of cattle meat/hectare can be obtained avoiding the soybean inclusion in the diet (Walker et al., 2013). Various economic, environmental and social challenges drive soybean production process (Jia et al., 2020), which has a negative impact not only during the production process itself but even considering the whole framework and hence its side activities. In much detail, the environmental impact and the costs required for the transportation to satisfy the worldwide demand is undeniably huge (He et al., 2019). Furthermore, the water requirement for soybean crops represents the umpteenth big issue (Eranki et al., 2019). Unsurprisingly, the animal feed produced covers 70% of the water and land consumption of soybean production (Steward, 2007). Additionally, deforestation (Ferreira et al., 2016) and biodiversity losses (Linhares-Juvena & Neeff, 2017) follow the previous exposed problems. Moreover, as a deforestation consequence, the reduction in carbon affects both the climate change and soil sources in terms of disposable water, sediment composition, and temperature (Macedo et al., 2012; Neill et al., 2013). To better understand the impact of this production, it is sufficient to think about 58% of carbon dioxide emitted by Brazil in 2005 (one of the main soybean producers) predominantly derived from deforestation and land-use conflicts than utilization of fossil fuels (Borzoni, 2011).

## 3. Quality over quantity: towards alternative poultry genotypes and poultry/avian biodiversity conservation

"*The management for human use of the biosphere so that it may yield the greatest sustainable benefit to present generations while maintaining its potential to meet the needs and aspirations of future generations. Thus conservation is positive, embracing preservation, maintenance,* *sustainable utilization, restoration and enhancement of the natural environment.*” (FAO, 2023). This reported above, represent the concept of natural resources conservation defined by the International Union for the Conservation of Nature and Natural Resource’s (IUCN) World Conservation Strategy. One of the main concerns related to high-production farm animals is the loss of genetic variability, related to the disuse of local breeds, less efficient and productive compared to the selected hybrids. Consequently to this mechanism, conservation issues arose in the last decades to become emergency of primary importance (FAO, 2023). However, face the breed conservation topic, includes discussing about sustainability and its several facets. The aspects which must be evaluated when the poultry sector is mentioned are various. Firstly, it is undeniable that medium and slow-growing chickens do not benefit of fast-growing efficiency. Therefore, they are less sustainable in terms of land use (major space for each bird), higher feed conversion ratio (Rezaei et al., 2018) longer rearing cycle, greater costs for bird maintenance (Tallentire et al., 2018) and lower performance (Fanatico et al., 2008). Moreover, due to the costs sustained by the farmers, an increase in the prices is unavoidable. On the other hands, the society is nowadays more prone to the ethic constraints (Lund et al., 2016) and fortunately, conservation of local genotypes cannot disregard high production standards, favoring the quality to the quantity. The conservation of local breeds should be interpretated as valorization of native products, strictly ingrained to cultural and traditional aspects proper of specific realities. Furthermore, many autochthonous breeds, by virtue of their cultural interest among population, may be conserved for their aesthetic features, as demonstrated by the amatorial rearing of several ornamental poultry breeds (FAO, 2023). Nevertheless, this sake in poultry breeds is still not enough to guarantee a sufficient protection from extinction. Additionally, the preservation of genetic biodiversity will be surely demanded in future, when different market necessities and external factors such as climate changes, will impact on new livestock population required. Animals resistant to disease and adverse environmental condition are essential, enhancing as well the peculiarities for what those breeds where originally typified (FAO, 2023). The support of cultural traditions and the linkage between consumers and lands would be thereby granted (Sponenberg et al., 2019).

*3.1 Current status of Italian poultry breeds*

As, mentioned above, chickens' population reared for meat and egg consumptions rapidly vary among years, thus the maintenance of alternative genetic options in poultry stocks is indispensable. The *in situ* conservation projects, despite being harder managed than cryogenic storage, represents a valid tool to protect endangered breeds (FAO, 2023). Castillo et al. (2021) provided a complete overview of the Italian poultry breeds status and current birds' presence on the territory, which revealed a scant Italian poultry breed heritage. A census questionnaire has been subjected to the farmers and the scarcity of some indigenous breeds has been clarified. Among the endangered ones, six chicken breeds has been revealed as particularly critic, with a count from 18 to 186 birds in total (Castillo et al., 2021; Franzoni et al., 2021). Numbers greater than 1000 chickens were identified for four breeds solely, to reach the maximum value of 3400 birds. The Bionda Piemontese (BP) and Livorno are the most popular ones, reared as a dual-purpose breed and an egg-laying hen, respectively. Some of the identified breeds were neither listed by breeders: the Collo Nudo Italiano, Pollo Trentino, and Tirolese, and Millefiori Piemontese as chicken breeds and the Castano Precoce turkey breed. The issues related to the protection of such breeds is partially related to the wide ignorance of both breeders and population. Campaign to promote and spread the information aiming at reaching the consumers is required to increase the current population size of some breeds. Collaboration among breeders, university research centers, and public entities is needed to reach such objective. Information, formation, and education are necessary both considering the knowledge in the existence of the animals themselves and considering the animal-derived products and the related culinary traditions. Incontestably, the consumers' palate developed a habituation towards commercial chicken properties. Thus, the indigenous meat sensory attributes and quality may be not appreciated or altogether may be disappointing for an inexperienced consumer (Pellattiero et al., 2020). For this reason, educate the consumer in terms of recipe preparations is fundamental, maintaining, simultaneously, a vivid linkage with the culinary traditions. Moreover, clever marketing strategies are needed to promote local breeds and the products obtained (Pellattiero et al., 2020).

*3.2 Current statuts of Piedmontese chicken breeds:*

The Avian Conservation Centre for Local Genetic Resources of the University of Turin (Italy) located in Carmagnola (TO), Italy, was convened in 2016 by the Italian Ministry of Agriculture and Forestry Policies to support the biodiversity conservation of the Piedmontese chicken breeds (Figure 2). Two breeds were reared when the conservation center has been established: the BP and BS. The center was originally equipped with:

- a hatchery room, provided of brooders for the breeder's reform at the end of the cycle (on average every two years);
- a brooding area to allot the chicks for the first weeks in a controlled environment in terms of temperature and humidity;
- a barn featured 24 pens and outdoor access for the rest of the cycle.

Finally, a subsequent facility has been dedicated to the local breeds conservation, whereas the first one has been destinated to the experimental trials. All the animals are subjected at first to a phenotypical selection to respect the breeds' standard and then divided in families, based on genetical analysis, thus distributed in different pens. The cockerels elected for the breeding program are finally chosen in order to maintain the widest genetic variability, being part of a wider reconstituent project on the Italian chicken breeds (Sartore et al., 2016; Cendron et al., 2020; Soglia et al., 2020; Soglia et al., 2021).

Currently, some research is in progress on a third Piedmontese breed which was considered as extinct: the Millefiori Piemontese. The breed is considered as a heavy genotype, characterized by an inconsistent white-black spotted plumage and red earlobes, observed for the last time in the 1990s (Agraria.org. Razze Polli: Millefiori Piemontese). The breed re-emerged thirty years later by virtue of small local breeders, who stated to own some specimens. By means of the collaboration among the breeders and the university, the first flock hatched in the spring 2023 and was allocated in the conservation center. The studies are still at the beginning, but the first genetical analyses showed the population as different from the Ancona breed, which has a similar plumage color but distinct genetic traits (unpublished data).

Figure 2. Main native Italian chicken breeds (Source: Razze - TuBAvI – Polli italiani).

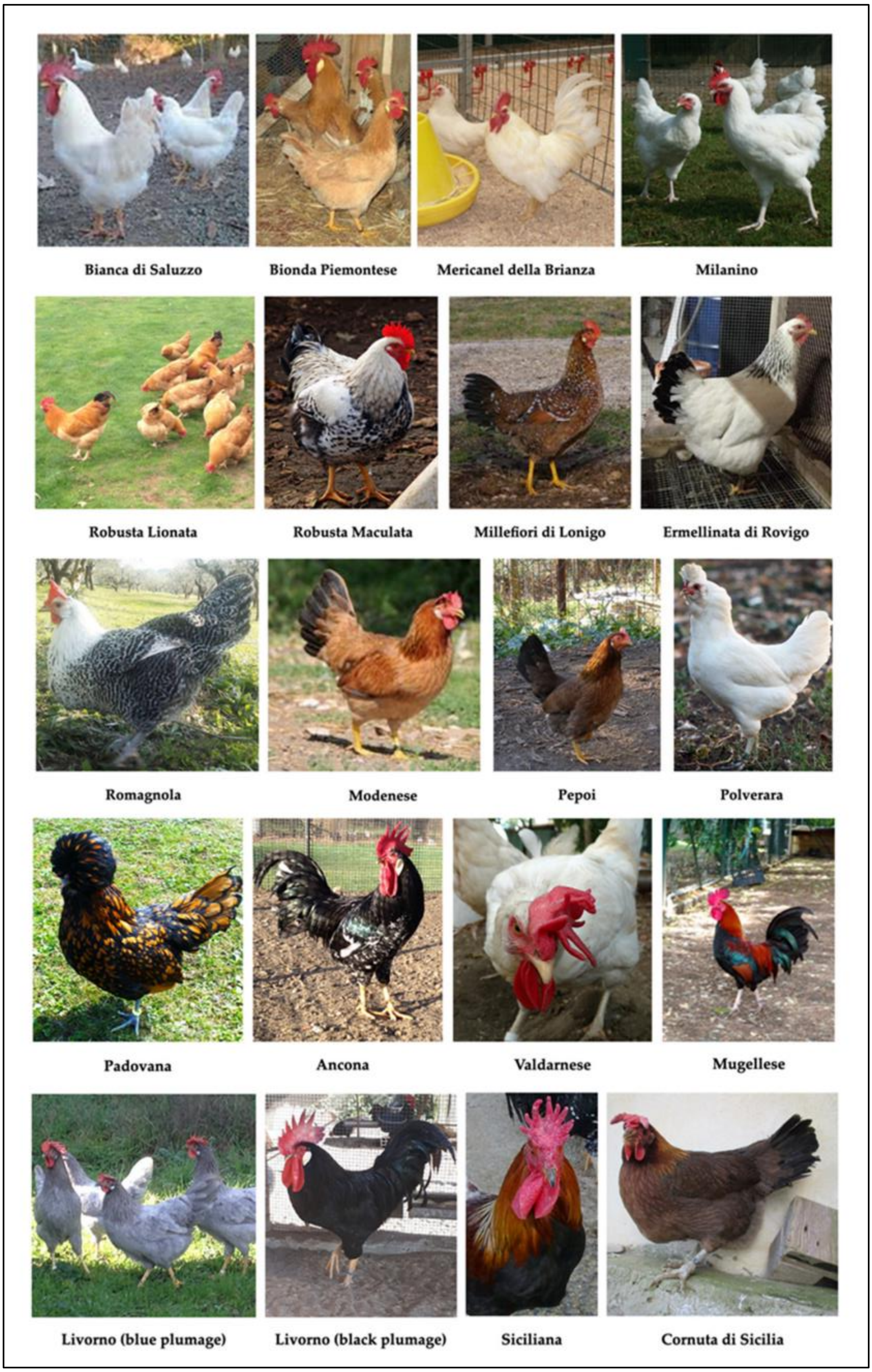


Figure 3. Avian Conservation Centre for Local Genetic Resources of the University of Turin and its chickens' population.

### *3.2.1 Bionda Piemontese*

The BP breed (Figure 4), know even as Bionda di Villanova, Bionda di Cuneo, Rossa delle Crivelle, or Nostralina, is present on the Piedmont territory since 1930. The first conservation and selection program were namely initiated in the 1938 by the Avian Experimental Center of Turin. In the post-war period, the breed has been neglected, due to the industrialization and introduction of the intensive agriculture. Finally, in 1999, the Professional Institute for Agriculture and Environment of Verzuolo (Cuneo, Italy) started a new conservation program to create standards ascribable to this breed, then approved in 2007 by the Italian Federation of Avian Associations (FIAV). Such breed characteristics encompass a buff-colored coat and black or metallic blue tail, with yellow to creamy-white earlobes, red wattles, and combs. The birds are characterized by medium dimension, strong conformation, and a F' production capacity of about 200 eggs/year. Usually slaughtered at approximately 180 days of age (first profitable slaughter period for the farmers), in some parts of the Piedmont region these animals are traditionally reared as seasonal products (consumed mainly during Christmas time), and for this kind of product the rearing period could be longer (up to 8 months) (Bongiorno et al., 2022). Now considered as not endangered breed, it has been part of the Slow Food Presidia until 2017 (TuBAvI – Polli italiani, 2023).

Figure 4. Mesmerizing males of BP.

*3.2.2 Bianca di Saluzzo*

The BS breed (Figure 5), known as well as Bianca di Cavour, has a white coloration characterized by spotted pale-yellow or cream feathers. The comb and wattles are red with yellow to white earlobes and yellow beak and feet. The medium dimension of this breed and the balance between the skeleton and muscular profile give it a high resistance to pathogens and adverse environmental conditions (De Marco et al., 2013). Moreover, the BS is even part of the Slow Food Presidia (Gallina bianca di Saluzzo - Presìdi Slow Food - Fondazione Slow Food), an organization which supports local food products linked to the respective territories and cultures, with supporters present in 150 countries all over the world. This breed was popular in the past between France the medieval marquisate of Saluzzo and Turin, especially in the surroundings of Cavour, renowned for its poultry market and traders from every Italian region. Typical dishes derive from its meat are the "*pollo alla cacciatora*" (portioned chickens cooked with tomato sauce) and "*tonno di gallina*" (hen cooked in vegetable broth and portioned to be pickled conserved), while the dish "*finanziera*" (dish prepared staring from animal waste:

combs, liver, wattles, kidneys cooked with other beef meat/organs, vegetables and Marsala wine). These breeds are thus suitable for a complete utilization of the animal parts, without waste, considering both sexes, as well as spent hens. Although the females are mainly reared for egg production (about 180 eggs/year) the spent hen is namely intended for boiled meat preparation or served with salad, representing another typical regional dish (Slow Food, 2013).

Figure 5. Nice males of BS breed.

4. Insects: a promising ingredient in poultry feed

Due to the global population increase, together with the raw materials costs and the deficiency of protein sources for feed production, alternatives and more sustainable assets are pressingly needed in substitution of soybean and fish meal (Lu et al., 2022). The use of insects as feed revealed an enormous potential over the past years of research and an expansion in their utilization is forecasted despite technical, financial, and regulatory constraints. Insect-based diets led to a major efficiency in the use of available nutritious sources, reason why several research has been conducted to include insects as feed ingredient and substitute part of the common ones (Shaviklo, 2023). Since insects are naturally part of animals' diet, their administration meets animals' feed acceptance (Sogari et al., 2019). Moreover, insects administration is promising in terms of nutritional composition and amino acid profile (Makkar, 2018), environmental sustainability with a reduction in the greenhouse gases produced (van Huis, 2013), lower surface required to obtain 1 kg of protein (Makkar, 2018), and the ability to convert low quality substrates into products with a higher biological value. Furthermore, the increase of population to 9 billion of people forecasted for the 2050 (Shaviklo, 2023), will dramatically boost the request for alternative ingredients for farmed animals (Khan, 2018) and about 1.6 billion tons of worldwide produced food is wasted yearly, although approximately 1.3 billion tons is still edible, accounting for $CO_2$ emissions equal to three times USA and China (Gligorescu et al., 2020). In this regard, the unsustainability of soybean must be stated, being considered as the most critical raw material in poultry diet. Its impact is related to deforestation, biodiversity losses, soil erosion, greenhouse gas emissions, water consumption and land-use conflict (Jia et al., 2020). The fish meal cannot be considered lesser impactful, due to the adopted fishing practices responsible for wild fish population depletion. Moreover, fish source are namely far located from poultry diet producers, representing a huge resources use for the raw material transportation, and supporting a mechanism more than in contrast with the circular economy (Fanatico et al., 2018). Furthermore, several studies presented the BSFL as potential partial replacement of fish meal included in poultry diet (Gligorescu et al., 2018), with CP values of defatted BSFL meal close to the ones obtained by meat and fish (El-Hack et al., 2020). As reported in the next chapters, the consumers' acceptance of insects will not have such a potential limitation in their use as feed protein ingredient. On the other hand, further research is needed to better understand the availability of consumers in paying products obtained by insect-fed animals, considering the consumers' sensory perception and the appeal of the final product obtained (Sogari et al., 2019).

### *4.1 Insects as food and feed: regulations among countries*

Various reality can co-exist on the use of insects as food and feed all over the world. On the basis of what reported by Khan et al. (2018) mealworm, housefly, BSF, earth worm, grasshopper, silk worm pupae, pallid emperor moth, cricket and locust are the most popular insects integrated in poultry diet. A description of the countries' allowances is reported in the Table 1 and paragraphs below (Sogari et al., 2019; Lähteenmäki-Uutela et al., 2021; IPIFF,2023), despite a greater homogeneity and cooperation among countries is needed compared to the current overview of the situation, in order to uniform the legislation, hence the use of insects all over the world (Veldkamp and Gasco, 2023).

Table 1. Current legislation in the matter of insects as feed use all over the world.

| Country | Authority | Regulation | Insects as feed |
|---|---|---|---|
| European Union | EFSA | European Union decisions/regulations | See Figure 6 for the current European legislation |
| USA | FDA | FFDCA | Additive approval list or GRAS needed for insects;<br>BSFL included as ingredient for animal food |
| Canada | CFIA | FAFR | Feed raw material needs authorization, HI product authorized for poultry |
| Mexico | SSA and SAGARPA | NOMs | no indication related to insects as food and feed |
| Brazil | Not indicated | Codex Alimentarius standards | No insects foreseen as feed |
| North Korea | Ministry of Agriculture, Food and Rural Affairs | Not present | Prohibited |
| South Korea | Ministry of Agriculture, Food and Rural Affairs | Not present | No authorization required |
| China | Ministry of health | Not present | Authorization required for novel feed materials added to the Feed Materials Catalogue |
| Japan | Ministry of Agriculture, Forestry and Fisheries | Act on Safety Assurance and Quality Improvement of Feeds | Importers and/or dealers must submit notification prior to starting a business |
| Thailand | Ministry of Agriculture and Cooperatives | Not present | Widely used |
| Australia | FIAAA | FIAAA Code of Practice and FeedSafe Standard | No registration for feed ingredients naturally part of animal diet. Insects may be used as aquaculture feed in every state, as poultry feed in NSW, ACT, Tasmania, Victoria, and Western Australia. |
| African countries | Not indicated | Not present | Great demand for insect protein and commonly used as food and feed |

EFSA: European Food Safety Authority; PAPs: Processed Animal Proteins; FDA: Federal Food and Drug Administration; FFDCA: Federal Food, Drug, and Cosmetic Act; GRAS: Generally Recognized as Safe; BSF: black soldier fly; CFIA: Canadian Food Inspection Agency; FAFR: Food Act and Feeds Regulation; SSA, Health Secretary; SAGARPA, Agriculture, Cattle, Rural Development, Fishing and Feeding Secretary; NOMs Normas Oficiales Mexicanas; NSW, New South Wales; ACT, Australian Capital Territory; FIAAA, Feed

Ingredients and Additives Association Australia; FIAAA Code of Practice, Australian and New Zealand Code of Practice for Animal Feed Ingredients and Additive Suppliers.

### *4.1.1 European Union: an insect legislation in constant evolution*

The European Union is responsible for the regulations of the insect producers, as part of the feed and food business operators, with the aim to commercialize the products obtained respecting the European Union standards. The general food and feed safety principles are gathered in the 'General Food Law' (Regulation N° 178/2002) and the 'Hygiene Package' (Regulation N° 852/2004 on the hygiene of foodstuffs and Regulation N° 183/2005 for feed hygiene) (IPIFF, 2023). On the bases of the above regulations, producers of insects must fulfil safety criterions of the obtained products at the distinct stages of the production activity. Restriction on the substrates which can be administered to insects are even applied, being insects considered as farmed animals (animals kept to produce food, feed, or other derived products). Only substrates of vegetal origin are allowed to be used as insect feed, despite milk, eggs and their products, honey, and non-ruminants' rendered fat or blood represent and exception. Finally, slaughterhouse or rendering derived products, manure, catering waste, and fish/meat derived unsold products from supermarkets or food industries are prohibited. Moving further, the insects' health and biosecurity must be guaranteed by the insect producers to prevent the spreading of diseases in the flock (EU Animal Health Law). However, being the insects non-vertebrates animals, there are no regulations so far regarding their welfare and farmers' compliances (Barrett et al., 2023). Nowadays, A total of seven species of insects are authorized (Regulation N° 2001/999 (Annex IV) amended by the Regulation N° 2017/893 (Annex X) for aquaculture, pigs and poultry feed (insect derived protein -PAPs- already admitted for aquaculture), became then eight with the Regulation (EU) 2021/1925 (IPIFF, 2023): BSF (*Hermetia illucens*), common housefly (Musca domestica), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*) and field cricket (*Gryllus assimilis*) and silkworm (Bombyx mori). Finally, the use of processed animal proteins (PAPs) was approved in poultry and pig feed by the European Union Member States (Regulation (EU) 2021/1925) (IPIFF, 2023). If dead whole insects untreated or treated are still forbidden in the European Union, live insects are instead allowed to be provided as feed, despite every state member has freedom of adopting national regulations against or in favor to use such products as feed (Figure 6) (IPIFF, 2023).

| Insects as feed - **Regulation (EU) No 68/2013** on the Catalogue of feed materials and in accordance **with Regulation (EC) No 999/2001 and Regulation (EC) No 1069/2009** | Ruminant animals | Aquaculture | Poultry | Pigs | Pets | Fur and other animals (e.g. zoo) | Technical uses(e.g. cosmetic industry, bio-based fuels, production of other bio-based materials such as bioplastics) |
|---|---|---|---|---|---|---|---|
| **Insect proteins** (under entry 9.4.1. 'Processed animal protein') | ✗ | ✓ ** | ✓ ** | ✓ ** | ✓ | ✓ | ✓ |
| **Insect fats** (under entry 9.2.1 'animal fat') | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| **Whole insects (untreated)** (under entry 9.16.2. 'terrestrial invertebrates, dead') | ✗ | ✗ | ✗ | ✗ | ✓ *** | ✓ *** | ✓ |
| **Whole insects (treated- e.g. Freeze drying)** (under entry 9.16.2.'terrestrial invertebrates, dead') | ✗ | ✗ | ✗ | ✗ | ✓ *** | ✓ *** | ✓ |
| **Live insects** (under entry 9.16.1 'terrestrial invertebrates, live') | ✗ | ✓ * | ✓ * | ✓ * | ✓ *** | ✓ *** | ✓ |
| **Hydrolysed insect proteins** (under entry 9.6.1. 'Hydrolysed animal proteins') | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

*if authorised by the national competent authority of the Member State where the product is being commercialised
** Limited to Black Soldier Fly (*Hermetia illucens*), Common Housefly (*Musca domestica*), Yellow Mealworm (*Tenebrio molitor*), Lesser Mealworm (*Alphitobius diaperinus*), House cricket (*Acheta domesticus*), Banded cricket (*Gryllodes sigillatus*), Field Cricket (*Gryllus assimilis*) and Silkworm (*Bombyx mori*).
*** if authorised by the national competent authority of the Member State where the product is being commercialised, under the specific conditions applicable to processed pet food (in case the product is intended for use as processed pet food)

| | |
|---|---|
| Restriction to insect species (insect PAPs for aqua feed)- Regulation (EU) **No 142/2011; Annex X Chapter 2 Section 1, A.(2).**<br>- Insect PAPs must be produced in **processing plants approved** in accordance with Article 24(1)(a) of Regulation (EC) No 1069/2009 and **dedicated exclusively** to the production of products derived from farmed insects '**Regulation (EC) No 999/2001; annex IV, chapter IV, Section F, 1 (a).**<br>- Insect PAPs must be produced according to **processing methods 1 to 5** or **processing method 7 (Regulation (EU) No 142/2011, Annex X, Chapter II, Section 1, B (2).** | (blue) |
| No restriction as to the insect species (provided that these are not pathogenic to humans and animals) | (yellow) |

Figure 6. Current insect products allowed and not allowed in the European Union (IPIFF, 2023).

*4.1.2 North America: the insect legislation and their inclusion as novel feed*

The Federal Food and Drug Administration (FDA) in collaboration with the Association of American Feed Control Officers (AAFCO) represent the authorities responsible for the animal feed safety in the USA, concerning feed regulation and novel feed ingredients (Lähteenmäki-Uutela et al., 2021). The edible insects can be found among the food additives admitted in the United States, despite solely the dried whole BSF and the BSF meal are allowed as feed ingredient and only in aquaculture. The Animal Feed Division, Animal Health Directorate, of the Canadian Food Inspection Agency (CFIA) dealing with the Food Act and Feeds Regulation of 1983, managing the admitted feed and feed ingredients in Canada (Lahteenmaki-Uutela et al., 2017). Since in this country insects are classified as novel feeds, they devoid of a safety-history regarding their use. For this reason, the authority's attention is centered on animal health and environment risk assessment, focusing on the species and the rearing steps required for the specific insect proposed for the registration. The BSF use in chicken has been authorized in 2016, to be extended to all poultry in 2018.

#### *4.1.3 Central and South America: no regulations but will in improve the insect as feed sector*

The use of insects as food and medicinal purposes was broadly diffuse in Mexico in the past. The regulation of the products which may represent a health threat are controlled by the Normas Oficiales Mexicanas (NOMs), mandatory standards which must be respected and are revised on a quinquennial base. So far, no indication related to insects as food and feed are present. On the other hand, movement towards the insect rearing have been observed in Brazil, where companies showed their interest in increases the sustainability of poultry products, by replacing the soya bean with the use of insects as feed. The regulation followed by this area is the Codex Alimentarius standards, based on what has been reported by Allegretti et al. (2018).

#### *4.1.4 Asia: contrasting scenarios among laws absence and prohibitions*

The concept of insects used as food and feed is faced differently in Asia. In numerous countries their inclusion is well accepted, being the insects a good source of protein. In China, the novel raw feed materials must be approved by the Ministry of Health and added to the Feed Materials Catalogue (Lahteenmaki-Uutela et al., 2017), whereas in Japan, feed suppliers must notify their activity to the Ministry of Agriculture, Forestry and Fisheries prior starting their business. Regarding Korea, defined legislative boundaries divide the territory. In other countries, some species of insects are traditionally mass-reared as feed and detailed protocols are already followed. For instance, Thailand is the greatest cricket producer in the world and own a Standard for cricket farming (Good Agricultural Practices for Cricket Farm, Thai Agricultural Standard 8202-2017), which foresees regulations on the farm management and facilities and animal health, aiming at obtaining good quality crickets. Although the preparation of this country in terms of animal feed legislation, for which the Ministry of Agriculture and Cooperatives regulation is responsible, indication about insects as feed are missing (Lähteenmäki-Uutela et al., 2021). The North (Democratic Republic of Korea) and the South (Republic of Korea) have namely an opposite overture to insects as food and feed. Since insects represent animal-based protein, the North Koreas regulation forbids the use of insects as feed (Jo & Lee, 2016). By contrast, the opposite is valid in South Korea, where insects are part of the culture as human and animal diet, despite no specific law are present after the deregulation of insect related law applied by the South Korean government in 2015 (Han et al., 2017). Recently, a review on the updated situation about insect as food and feed has been provided by Kwon (2022) from the South Korea. Since 2011, various governmental funds were dedicated to the research, despite the approach to the industrial sector has not been completed yet. Most farmers are represented by small holders in Korea, although the 300% increase in the number of insect farmers, with an ongoing development of the insect industrialization legislation. Failures in the Korean government can be attributed to the facilitation of insect use spread, such as the allowance in the use of insects as food source (Kwon, 2022).

*4.1.5 Oceania: what is naturally present in animal diet is allowed to be used as feed*

Considering Australia, feed ingredients and additives provision are regulated by the Feed Ingredients and Additives Association Australia (FIAAA) by means of the Australian New Zealand Code of Practice for Animal Feed Ingredients and Additive Suppliers (the FIAAA Code of Practice) and the FeedSafe Standard (https://fiaaa.com.au/about/#bg). Nonetheless, this country does not require a registration for feed ingredients naturally part of animal diet, despite the strict legislation regarding bovine spongiform encephalopathy. Meat, meat and bone meal derived from all vertebrates, including fish and birds represent namely forbidden ingredients for ruminants. Moreover, the use of insects is limited to aquaculture in all states, whereas it is allowed as poultry feed in New South Wales, Australian Capital territory, Tasmania, Victoria, and Western Australia. Precise rules are defined for the use of insects as feed. They cannot be fed with meat, manure and catering waste, similar to Europe, whereas the use of live and heat-untreated insects is forbidden in Australian territory (DiGiacomo et al., 2019). On the bases of the information provided by the Institute Public Administration Australia, research aimed at developing the insect sector is conducted by five major commercial fly farms and various small-scale farmers.

*4.1.6 African countries: insects among traditions and development of industrial-scale production*

Africa is characterized by a huge insect biodiversity and a surprising request of insect protein with the objective to replace the food and feed importation and create a local job network which can guarantee an income. The territory is characterized by an inconstant regulation pattern with no specific laws related to the insect prodcution. In South Africa, the insects as food are regulated by various organs, but the legislative framework lacks of indication related to insects used as feed, despite the presence of emerging industries, which already produce insects as feed at a commercial scale, focusing in particular on the BSF production as monogastric feed ingredient (Niassy et al., 2018). Similarly, a lack of information is described by Nakimbugwe et al. (2021), in the Sub-Saharan countries in general, with no official regulations from governments, although insects represent a natural component in terms of both food and feed in those countries and the abundance of projects designed at establish policies on the insect use. In countries like Nigeria, Coleoptera, Isoptera and Hymenoptera, are considered as food and the indigenous experience on insect harvesting combined with the research could lead to the development of this sector on an industrial scale (Lähteenmäki-Uutela et al., 2021; Usman and Yusuf, 2020).

*4.2 Insects as feed? The floor to the consumers*

Although a various range of studies is available regarding the consumers' appreciations and preferences towards the use of insects as food (Sogari et al., 2019), little information is disposable regarding the consumers' acceptance of insects as feed and derived products. The PROteINSECT European project carried out two surveys between 2013 and 2014, and in 2015, revealing a general approval among population in the use of insects as feed for farmed animals (Sogari et al., 2019). Interestingly, 64% of participant did not show health concern regarding the topic, while 88% asked more questions, demonstrating interest and curiosity, highlighting even a lack of information and formation in the matter (Sogari et al., 2019). In Belgium, among various farmers, agricultural stakeholders, and citizens, a higher level of acceptance in the use of insects especially as fish and poultry feed has been overall observed (Verbeke et al., 2015). Two surveys were instead conducted in 2016 in Italy (Laureati et al. 2016; Mancuso et al., 2016). Laureati et al. (2016) considered a population sample made of Italian students and employes from the University of Milan, and respondents unconnected to the academic field, in order to compare the different consumers' perspectives regarding the use of alternative meal for reared animals. As a results, 53% of the interviewed were amenable to the inclusion of insects in livestock's diet, maintaining the same opinion in eating animals reared that way.

Similar results were gathered in other research conducted in Poland (Kostecka et al., 2017) and United Kingdom (Popoff et al., 2017). Similarly to the study carried out by Verbeke et al. (Verbeke et al., 2015), poultry were the preferred species in which propose insect inclusion in diet (58.1% of the interviewed), followed by fish (56.7%). By contrast, a retention in the consumers' consumption of products obtained was observed comparing these species with cattle and pigs. The most conceivable explanation is reconducted to the natural evolution of these species, being the insects commonly present in birds and fish diet both in the wild and in free-range or organic systems. In addition, the attitude towards insect inclusion in animal diet could be sponsored and encouraged by means of public communications and events created for the promotion of such products, enhancing their sustainability in comparison with products derived from traditional feeding systems (Sogari et al., 2019).

## *5. Black soldier fly*

### *5.1 Overview: life cycle and general traits*

The BSF, which origins are neotropical, had the capability to propagate through the entire American continent, preferring temperate-tropical climates with optimal condition between 27-30°C (Sheppard et al., 1994; Sheppard et al., 2002; Marshall et al., 2015). Furthermore, due to its climate adaptability and flexibility (in terms of light, temperature, and humidity), and due to antrophization, it reached Australia, India, Africa, and Europe (Gujarathi & Pejaver, 2013; Martínez-Sánchez et al., 2011). Undeniably, at present, the BSF represents one of the most auspicious insects suitable for animal feed purposes, by virtue of its prominent food waste bioconversion capacity. BSF is a holometabolous insect, belonging to Diptera species. The rearing cycle of BSF is composed by various phases, which comprehend the egg, the larvae, the prepupae, the pupae and the adult fly (Figure 7). Two days after the metamorphosis, the mating starts among males and females,and on average five hundred white-creamy eggs are laid by the adult females (Diclaro & Kaufman, 2009) closed by decaying organic substrate, essential for the growth of the larvae from the hatching till the prepupae stage. The eggs hatch after 4 days and the maximum length reachable by the larvae is 27 m and 6 mm in width, with a white-pale yellow coloration and a chewing mouthpart. A total of six larvae instars (about 14 days) are part of the cycle (Hall & Gerhardt 2002), which will eat to store enough nourishment for the other phases, in which the insects will not eat (Newton et al. 2005b), despite recent studies revealed better performance in adults provided with sugary water or honey (Barragan-Fonseca et al., 2017). Afterwards, the prepupae migrate toward sheltered and dried vegetation, to allow the initiation of the pupation phase, characterized by darkening exoskeleton. The pupae phase requires approximately two weeks for the adult emergency (Tomberlin & Sheppard 2001; Hall and Gerhardt 2002). Subsequently, the life duration of the adult last for about 8 days, for a total duration of the cycle of approximately 45 days. The bioconversion capacity of BSF is impressively high, with a feed conversion ratio ranging between 1.7 and 3.6 on DM (Gligorescu et al., 2020), reason why has been effectively employed to convert low-value materials in protein with a prestigious biological value expendable in animal feed sector (Barragan-Fonseca et al., 2017; Gold et al., 2018; Lalander et al., 2019; Makkar et al., 2014; Singh and Kumari, 2019; Wang and Shelomi, 2017). The BSF can indeed be fed a wide range of side stream, such as abattoir waste, food waste, human feces and a mixture of abattoir waste, fruits and vegetables, biogas digestate, manure, and municipal waste (Gold et al., 2018; Lalander et al., 2019; Singh and Kumari, 2019), despite as mentioned previously, not all the substrate are allowed in the European Union. Based on this peculiarity the BSFL have been successfully used not only to degrade food waste but even to expend swine and poultry manure in commercial farms (Newton et al., 2005b; Diclaro & Kaufman, 2009). Moreover, the greater sustainability of BSF compared to other protein source, such as fishmeal, has been demonstrated in different studies (Smetana et al., 2019). Discussing the use of this insect as feed, its utilization has been broadly debated both for poultry, swine and in aquaculture (Barragan-Fonseca et al., 2017; Makkar et al., 2014; Wang & Shelomi, 2017).

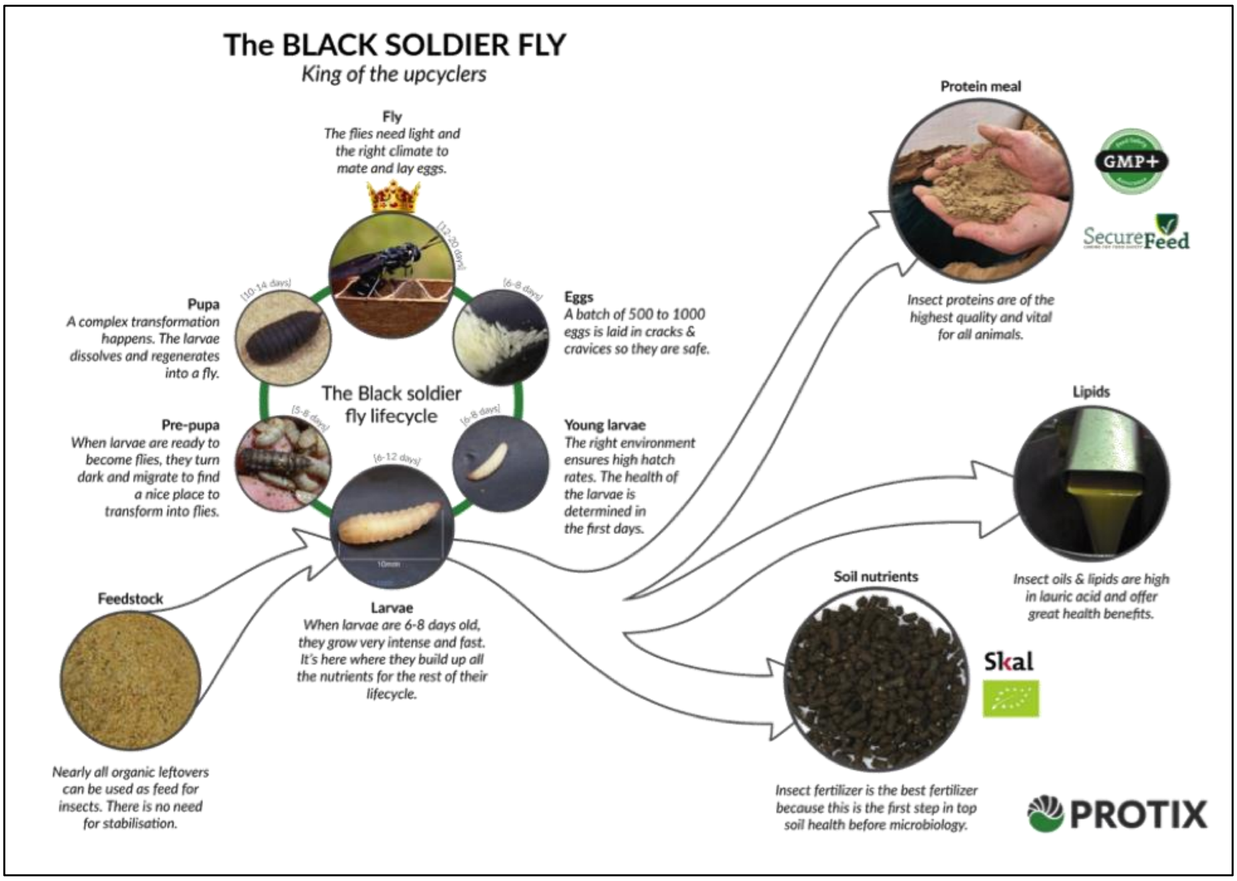



Figure 7. Black soldier fly rearing cycle (Source: Protix, Bergen op Zoom, The Netherlands; ).

## 5.2 Nutritional aspects and larvae processing

Overall, insects can be a valid alternative to soybean by virtue of their fat and protein content (30-40% and 40-60% on DM bases, respectively) (Khan et al., 2018). In particular, BSF is rich in protein (from 37 to 63% DM), and fat (from 7 to 39% DM) (St-Hilaire et al., 2007; Zheng et al., 2012; Barragan-Fonseca et al., 2017), based on the instar (to late ones correspond high DM) and the substrate on which the larvae are reared. The DM values range from 90 and 93%. The content of ash varies between 9 to 28% DM, while the lysine represents the most abundant amino acid among the ones present in larvae (6-8% of protein content) (Sheppard et al., 2008). To make a comparison with the soybean meal, BSFL are richer in alanine, methionine, histidine, and tryptophan, whilst they contained less arginine (Barragan-Fonseca et al., 2017). Regarding the fatty acid profile, the saturated fatty acid proportion amounts to 58%-72% and 19-40% monounsaturated and polyunsaturated fatty acids (on the total lipidic fraction) (Li et al., 2011; Kroeckel et al., 2012; Makkar et al., 2014), with greater level of lauric, palmitic and oleic acid (Surendra et al., 2016). Moreover, the fat contained in high-fatty diets is mostly turn into lauric acid, make challenging the modulation of fatty acids profile contained in the larvae (Oonincx et al., 2015b). On the other hand, the lauric acid is even well known for its antimicrobial properties, having a beneficial effect on the immune system (Fortuoso et al., 2019; Londok & Rompis, 2019). The mineral composition of BSFL encompasses relevant levels of manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), phosphorus (P) and calcium (Ca), present in a greater proportion than other insects and that could vary on a substrate used base (Dierenfeld & King, 2009; Chia et al., 2020) withtin a range of 2%-10% DM. Nevertheless, the digestibility and availability of nutrients should be improved in further studies, focusing on nutrient composition and larvae processing method used. Factors as substrate provided to the larvae, amount provided, and environmental drivers, such as temperature, moisture, and larvae density can affect the larvae performance and chemical composition. Undeniably, the wide variation of protein and especially fat composition depends on the substrate used to feed the larvae, in terms of quantity and quality (Newton et al., 2005a; Nguyen et al., 2015). The technological treatment on the larvae can affect their composition as well. For instance, Zulkifli et al. (2022) showed that spry-dried larvae displayed a higher content of amino acids than the oven-dried larvae, and the saturated fatty acids were more abundant than unsaturated ones in oven-dried larvae. Additionally, variation in the larvae composition occurs within the larvae life stages, with a protein content reduction proportional to the larvae growth (5 days old, 61%; 15 days old, 44%; 20 days old, 44 %, while, on the other hand, a higher DM characterizes the later instars. By contrast no great variations are reported in the amino acid profile (Barragan-Fonseca et al., 2017). In the Figure 8 the processing path of BSFL is depicted. The chitin is another important component in the BSFL. It is a linear polysaccharide of the amino sugar N-acetyl glucosamine, highly present in disparate invertebrates such as sponges, mollusks, nematodes, arthropods, and fungi (Moussian, 2019). This component covers a primary role in the external protection of these organism. Generally, chitin is an important component of protective or supportive extracellular matrices that cover the tissue that produces it or the whole body of the organism. Moreover, being the chitin a pliable extracellular polysaccharide, defines the mechanical characteristics of tissues, once combined with proteins (Moussian, 2019). In the insect regard, chitin is a component of the BSF exoskeleton, capable of immunomodulatory effects in mammals (Komi et al., 2018), and antimicrobial peptides with a chitin content up to 9% (De Souza Vilela et al., 2021). In the Table 2 the main composition of BSF is reported

Figure 8. processing path of BSFL and derived products (Shaviklo, 2023).

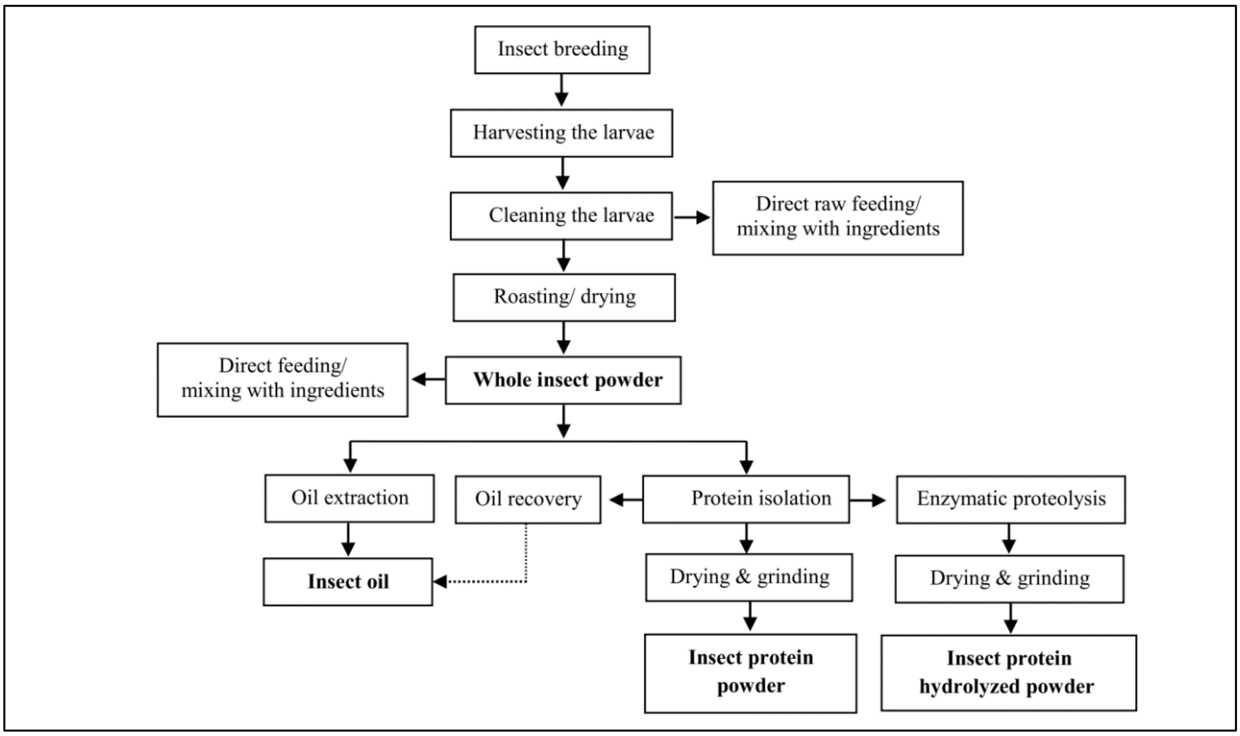


Figure 9. Black soldier fly phylogenesis starting from holometabolous insects (©Grimaldi and Engel, Evolution of the Insects, Cambridge University Press).

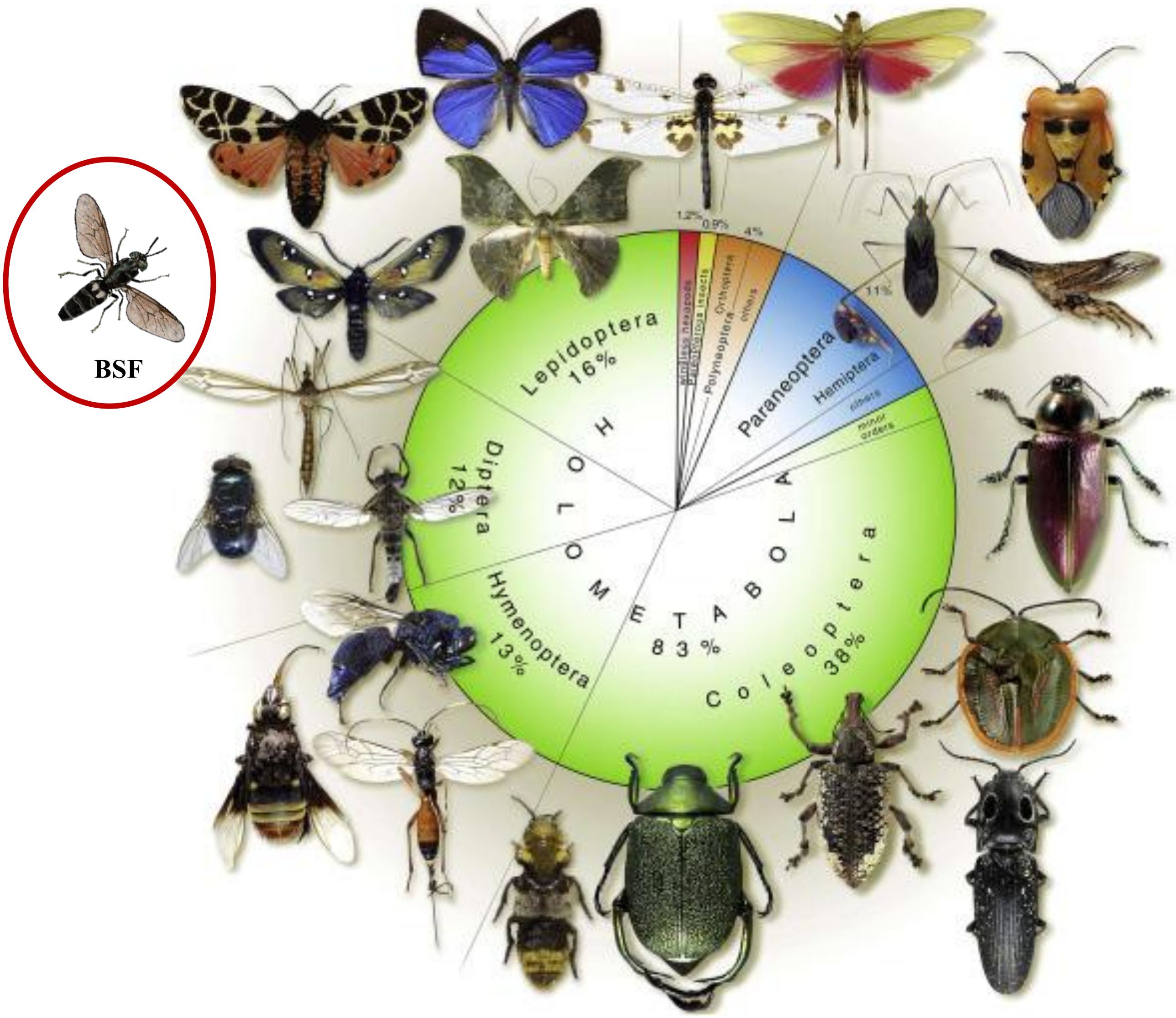


Table 2. Main composition of black soldier fly larvae

| Item | Unit | Average | Min. value | Max. value | Literature |
|---|---|---|---|---|---|
| Dry matter | % DM | 10 | 8 | 12 | (Chia et al., 2020) |
| Ash | % DM | 18.5 | 9 | 28 | (Sheppard et al., 2008) |
| Crude protein | % DM | 50 | 37 | 63 | (Barragan-Fonseca et al., 2017; Queiroz et al., 2021) |
| Ether extract | % DM | 23 | 7 | 39 | (Barragan-Fonseca et al., 2017; Queiroz et al., 2021) |
| Saturated fatty acids | % total LF | 65 | 58 | 72 | (Li et al., 2011; Kroeckel et al., 2012; Makkar et al., 2014) |
| Mono-polyunsaturated fatty acids | % total LF | 29.5 | 19 | 40 | (Li et al., 2011; Kroeckel et al., 2012; Makkar et al., 2014) |
| Chitin | % DM | 15 | 5 | 25 | (McKay, 2018) |
| Gross energy | MJ/kg DM | 6 | 5 | 7 | Bellezza Oddon et al., 2021 |
| Lysine | % of CP | 7 | 6 | 8 | (Sheppard et al., 2008) |
| Minerals | % DM | 6 | 2 | 10 | (McKay, 2018) |

Abbreviations: DM, dry matter; LF, lipid fraction; CP, crude protein; Max., maximum; Min., minimum.

## 6. Poultry production and BSF: a multifaceted opportunity

### *6.1 BSFL meal influence on birds' performance*

Various results are available regarding the use of BSF as feed ingredient and soybean substitution in poultry diet, despite more data are disposable on the use of the BSFL meal than the live larvae (Dörper et al., 2021). Most studies were conducted on broiler chickens and laying hens, despite publications are available on other species including turkeys, ducks, and quails. Moreover, the BSFL meals employed embedded full-fat, partially defatted, and defatted meals (Dörper et al., 2021). Starting from broiler chickens, the data gathered are inconsistent. Attivi et al. (2020), highlighted a reduced body weight gain at 2%, 4%, and 6% inclusion of full-fat BSFL meal in broiler chickens (reared from 14 to 56 days of age), while an ameliorated feed intake and feed conversion ratio, without thwarting the final body weight of the birds, was observed at 6% and 8% of inclusion. Positive effects on the body weight gain have been observed instead at 8% inclusion. Similarly, Dabbou et al. (2018), declared an increase in the feed intake and average daily gain of birds (reared from 1 to 35 days of age) during the first rearing phase, with not beyond 10% partially defatted BSFL meal inclusion, used in substitution of soybean meal, soybean oil and gluten meal. The hematological parameters were even consistent with the physiological range available in literature (Dabbou et al., 2018; Elahi et al., 2019). However, over this inclusion (until 15%), the broiler chickens performance resulted instead encumbered, with a lower live weight during and finisher period, together with a worse feed conversion ratio. Additionally, bacterial heterogeneity in the caeca and a inhibition of intestine mucin production has been detected at those level (Biasato et al., 2020). An inclusion of 0%-5%-10% and 15% was considered in the study conducted by Schiavone et al. (2019), in which broiler chickens were reared from 1 to 35 days of age and fed defatted BSFL meal in replacement of soybean meals, soybean oil, and corn gluten meal. By contrast, recent research reported a maximum inclusion of full-fat BSFL equal to 20% in the diet, which can guarantee an equitable broiler chicken diet, without impairing the performance and health of the birds. Moreover, if this percentage decreases at 15%, a positive effect on the immune system can be observed, by lowering the energy required by the immune system (De Souza Vilela et al., 2021). Other studies conducted on laying hens, reported no negative implications on feed conversion ratio and laying performance, in the partial (50%) or full replacement of soyabean meal by BSFL one, when related to an organic common diet (Maurer et al., 2016). In addition, De Marco et al. (2015) reported how the good apparent metabolizable energy and the amino acid apparent ileal digestibility coefficients of BSFL meal, should warn the feed industry about the treasured utilization for diet formulation in broiler chickens feed. Contrastingly, Attivi et al. (2020), observed a decrease in the protein digestibility at any inclusion of BSF, with a reduction in the gizzard weight and intestinal length. Finally, Martínez Marín et al. (2023)

ideated a regression model applicable for the estimation of the apparent metabolizable energy in BSFL meal, which could be relevant for future improvement in this sector. Other research is available on Muscovy duck reared from 3 to 50 days of age (Gariglio et al., 2019) with no negative performance implications observed for 9% inclusion of partially defatted BSF meal substituting corn gluten meal (0-3%-6%-9% of inclusion). Comparingly, no changes have been seen in Japanese quails reared from 10 to 28 days of age fed 0 to 10% BSFL meal in alternative to conventional protein and fat sources (Woods et al., 2019). In this research, the BSF was reared for one treatment 100% on layer mash, while for the second one 50% on layer mash and 50% on fish offal. However, the gross and metabolizable energy of BSFL reared on 100% on layer mash boasted greater digestibility than control groups, while a worsened body weight gain were recorded in the BSFL raised 50% on layer mash and 50% on fish offal than controls. Nonetheless, finding the right balance of BSFL meal in poultry diet remains an unsolved query, being involved several environmental and management factors, and meal processing and properties, which make challenging providing a defined percentage inclusion in poultry diet (Dabbou et al., 2018).

### *6.2 Poultry rearing and live BSFL: an intriguing tool to improve growth performance and welfare of the birds*

During the past three years the research made huge progress in the BSF sector, enriching the disposable knowledge, thus started exploring the use of these insects as a tool to improve birds' welfare. Therefore, the idea to provide live BSFL as environmental enrichment, considering even the effects on the performance and health of the birds, became firstly reality in a trial conducted on turkeys by Veldkamp & van Niekerk (2019). Afterwords, Ipema et al. (2020a) provided to broiler chickens two or four times per day 5% and 10% live BSFL based on the daily feed intake, with no influence on the other parameters, while Bellezza Oddon et al. (2021) found a major relative weight of spleen in broiler chickens fed 5% live BSFL (from

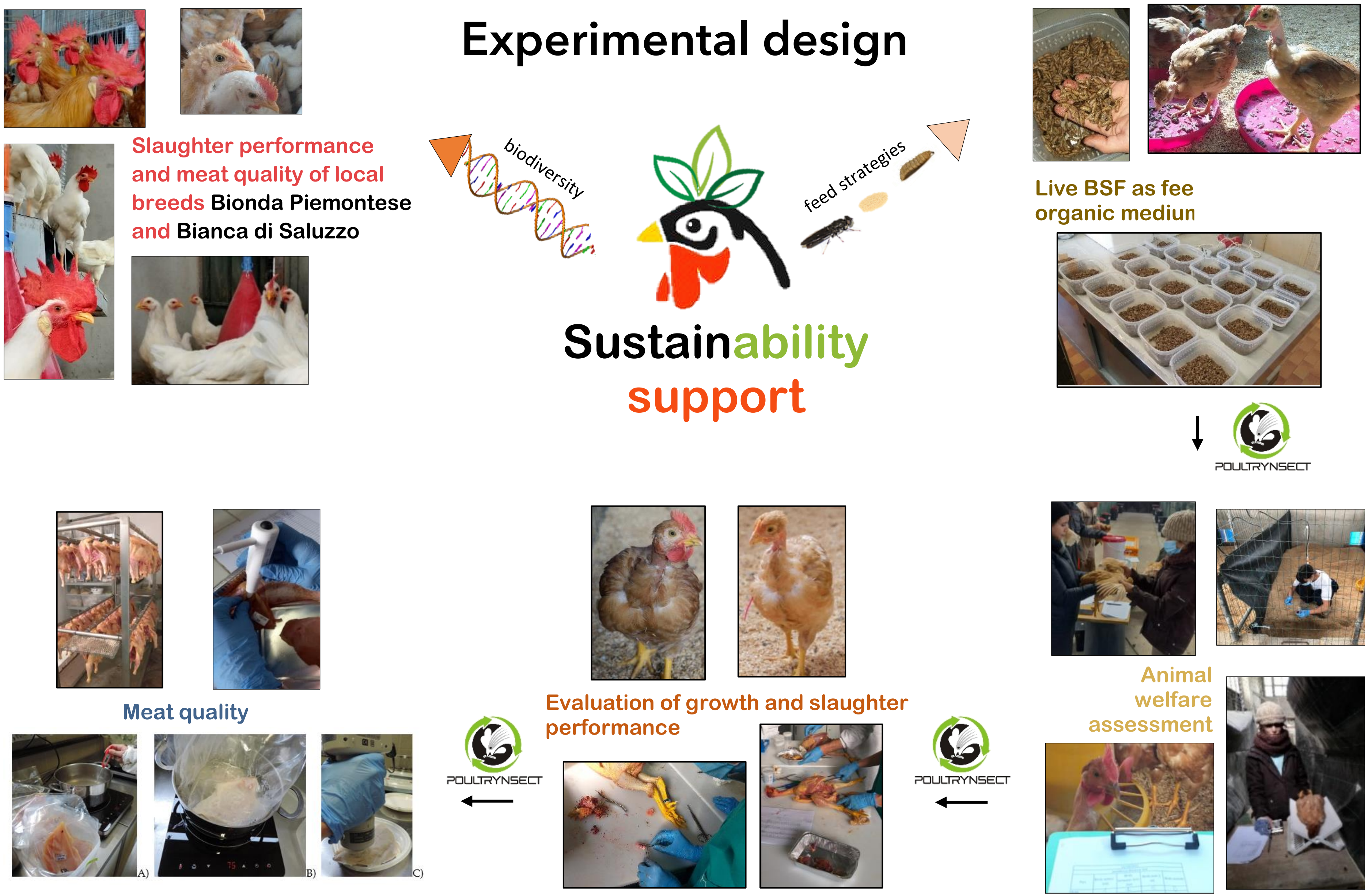
4 to 39 days of age) compared to the control groups. Finally, Ipema et al. (2022) replaced 8% of the DM in the diet with live, dried or meal of BSF from 1 to 35 days of age of the birds. They proved the live larvae scattering as the most efficient and functional method to maintain broiler chickens active, followed by the scattering dried larvae, rather than using a distributors or feeders. The foraging behavior during the whole cycle namely increased in the supplemented birds, complemented by a reduction in resting behavior in favor to standing idle and walking at the end of the rearing period. Moving to the laying hens, Star et al. (2020) provided live BSFL in substitution to soybean meal and nor the feed intake, feed conversion ratio, body weight, laying rate, egg weight, egg mass, egg quality parameters, neither the mortality displayed affection by the supplementation. Finally, a lower feed conversion ratio, improved feed intake and body weight gain were detected in turkeys fed 12% live BSFL in substitution to soybean meal, from 1 to 35 days of age (Veldkamp & van Niekerk, 2019).

The sustainability concept must be applied not only to the production itself but also to the animal welfare, in order to satisfy good welfare standards and guarantee the health of the flock. The degree of welfare encompasses ethic, productive, and commercial standards which are linked one to each other and must be achieved and maintained overtime. Primarily, poultry can experience pain and suffering in relation to inadequate welfare and health conditions. Therefore, poor rearing conditions led to a reduced production, with a reflection on the farmers' profit. Moreover, as reported in the overall discussion, the welfare conditions sway consumers' purchase decisions, affecting thereby the product demand (FAO, 2023). An increase in alternative rearing systems, promoting local breeds and slow-growing chickens, has been observed due to arising welfare and environmental issues noticed in intensive rearing systems, such as a reduction in the birds' activity and resistance against diseases, an increase in dermatitis and ascites prevalence, leg weakness, and overall a greater mortality incidence (Wallenbeck et al., 2016). Although the mentioned negative aspects the fast-growing systems are anyway the most abundant in Europe (90%-95%) (Augère Granier, 2019; Van Horne, 2018). Good welfare conditions can be achieved following good management practices and choosing alternative hybrids or local breeds which are less prone to broiler chickens' health issues. Furthermore, the introduction of environmental enrichments covers a relevant role in guaranteeing the welfare of the birds. In this thesis, the promotion of local chicken breeds and the combination between the use of an alternative genotypes and live BSFL as environmental enrichment are enhanced. Nevertheless, alternative rearing systems like organic and free-range should not be consider as equal to sustainable systems (embracing the sustainability concept mentioned above), inasmuch the production and welfare outcomes relies on good management practices (Van De Weerd et al., 2009; Riber et al., 2018). The major challenge is combining exploitable environmental enrichment with poultry strain efficiency, in terms of production and utilization of resources. In addition, a relief of the feed producers is needed and could be met by proposing and introducing greater environmental friendly protein ingredients than what currently available and mentioned above (Dörper et al., 2021). For the above-mentioned reasons and renowned market necessities, the use of live insects is an ideal solution which could satisfy the different sector enquires (Carr et al., 2016; Gasco et al., 2020; Purkayastha and Sarkar, 2022). Unlike the common environmental enrichment, including moss-peat shavings and wood, sand, and rice husk, live larvae present the prestigious attribute of being mobile, increasing the attraction of the birds and reducing them to perform one of the most ancestral stimuli: foraging and more generally, explorative behavior (Clara et al., 2009; Riber et al., 2018; Star et al., 2020). In the broiler chickens investigated in the study of (Ipema et al., 2020a) a greater ground pecking and overall foraging activity were observed in all the live larvae supplemented groups (the larvae were distributed two or four times per day 5% and 10%). Moreover, ameliorations in the hock burn score were detected in the live BSFL provision two and four times/day, compared to the control groups, whit higher gait score in control groups than the groups in which 5% of larvae were provided 4 times/day, or 10% both two and four times/day. Furthermore, Ipema et al. (2020b) noticed a broadly higher activity and more foraging displayed, in broiler chickens fed 5% or 10% live BSFL. Similarly, Biasato et al. (2022) found 5% live BSF provision in broiler chickens as functional at increasing their activity and foraging behavior. Moreover, the administration of larvae in this research resulted proficient in contrasting birds' fear as well. Nevertheless, no changes in the excreta corticosterone metabolites of heterophile/lymphocyte ratio were detected (Bellezza Oddon et al., 2021; Biasato et al., 2022). Finally, it has been demonstrated that live or dried BSF had a beneficial effect on the production performance of the birds compared to the control groups (Ipema et al., 2022). Regarding the laying hens, a reduction in feather pecking was recorded in white laying hens fed live BSFL in substitution to soybean meal from 67 to 78 weeks of age in the study conducted by Star et al. (2020). Curiously, the number of eggs laid on the floor in the morning was greater than in the afternoon, moment in which the live larvae were distributed. Tahamtani et al. (2021) focused their research on white laying hens administered 10%, 20%, or *ad libitum* live BSFL with no changes in birds' behavior. Veldkamp & van Niekerk (2019), reared turkeys until 35 days of age and fed 12% live BSFL. Despite the low occurrence of skin and plumage damage, they noticed a reduction in feather pecking, resulting in an improvement in plumage condition of the back and tail of the birds at the fifth week. By contrast, an increase in feed pecking during the first week was observed, while a reduction of feed pecking and drinking was reported at the third week of age. Finally, the pecking object frequency decreased during the fifth week.

Figure 10: live BSFL provision to a medium-growing genotype (picture taken during our trial).

## 7. Objectives of the research

The main aim of this thesis is the implementation of poultry production sustainability. Two factors affecting such multifactorial issue are discussed in the following chapters: promoting the use of local chicken breeds in order to support both environmental sustainability and genetic biodiversity, together with the employment of alternative feed ingredient which can reduce the soybean inclusion in chickens' diet. It needs to be specified that for this research we worked both with birds slaughtered at 7-8 months of age and at about 3 months of age. For this reason, even if a slaughter age of 82 days identifies internationally a slow-growing broiler, we will consider it as a medium-growing hybrid, in order make possible the comparison with our slow-growing genotypes BS and BP.


Experimental design
Slaughter performance and meat quality of local breeds Bionda Piemontese and Bianca di Saluzzo
biodiversity
feed strategies
Sustainability support
Live BSF as fee
organic mediun
POULTRYNSECT
Meat quality
A)
B)
C)
POULTRYNSECT
Evaluation of growth and slaughter performance
POULTRYNSECT
Animal welfare assessment

## 8. Carcass Yield and Meat Composition of Male and Female Italian Slow-Gro wing Chicken Breeds: *Bianca di Saluzzo* and *Bionda Piemontese*

*animals*

Article

# Carcass Yields and Meat Composition of Male and Female Italian Slow-Growing Chicken Breeds: *Bianca di Saluzzo* and *Bionda Piemontese*

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**Simple Summary:** *Bionda Piemontese* and *Bianca di Saluzzo* are two slow growing breeds from north-west Italy, specifically from the Piedmont region. Their low input requirements make them suitable in organic and free-range rearing contexts for both meat and egg production. This research, part of a conservation program for these two breeds, aims to define the meat properties and qualitative attributes of these two breeds, comparing them at different slaughter ages in order to identify the most profitable slaughter period. The results show significant benefits associated with slaughtering at 7 months of age, which outperformed the shorter rearing periods in terms of both better slaughter performances and meat properties.

**Abstract:** The slaughter performance and meat quality of two native Italian chicken breeds, *Bionda Piemontese* (BP, $n = 64$) and *Bianca di Saluzzo* (BS, $n = 64$), were investigated. Two-way ANOVA, considering breed, sex, and their interaction, was used to compare the properties of birds slaughtered at 5, 6, 7, and 8 months of age. Subsequently, data were analyzed using one-way ANOVA and the Duncan test to evaluate the differences between slaughter ages. The BP breed produced a better carcass yield than BS at 5, 7, and 8 months of age ($p < 0.05$). Breast moisture and crude protein contents were influenced by gender, and were higher in males than in females ($p < 0.05$). By contrast, the crude fat content was higher in females than in males ($p < 0.05$). The saturated fatty acid content of breast meat increased as the birds aged in both breeds ($p < 0.05$). The polyunsaturated fatty acid content of both breast and thigh meat was higher in males than in females ($p < 0.001$ and $p < 0.05$, respectively). In general, slaughtering at 7 months was associated with the best slaughter and meat quality characteristics in both breeds. Moreover, from a nutritional point of view, the characteristics of the meat from male birds were preferable to those of meat from females.

**Keywords:** chicken breed; meat quality; sex; carcass yield; fatty acid



**Citation:** Bongiorno, V.; Schiavone, A.; Renna, M.; Sartore, S.; Soglia, D.; Sacchi, P.; Gariglio, M.; Castillo, A.; Mugnai, C.; Forte, C.; et al. Carcass Yields and Meat Composition of Male and Female Italian Slow-Growing Chicken Breeds: *Bianca di Saluzzo* and *Bionda Piemontese*. *Animals* **2022**, *12*, 406. https://doi.org/10.3390/ani12030406

Academic Editor: Avigdor Cahaner

Received: 30 December 2021
Accepted: 7 February 2022
Published: 8 February 2022

## 1. Introduction

Over the last forty years, the preservation of animal genetic resources has become a matter of great concern, so much so that it was identified by the Food and Agriculture Organization of the United Nations (FAO) as one of its main objectives [1]. Since 1980, technical programs have been developed to gather global census information on all existing livestock breeds and to further activities focused on biodiversity in the livestock sector [1].

The use of just a few commercial poultry hybrids suitable for intensive rearing systems has led to a loss of the 90% of the alleles specific to local poultry breeds, the protection of which has consequently become an argument of great consequence [2,3]. In addition, the conservation of genetic variability is useful for preserving genetic traits that could be successfully used to satisfy future requirements, such as environmental and climate resistant traits [4]. From a commercial point of view, each local poultry breed presents distinct meat qualities, such as, for example, the highly tender and dark meat obtained from the *Padovana* breed. Furthermore, such genotypes are strongly associated with the specific local areas where they have historically been reared, becoming a distinctive social and economic symbol, and reinforcing the concept of poultry biodiversity conservation [5].

According to the Domestic Animal Diversity Information System, a database platform developed and managed by the FAO, 6.74% of the 1482 recognized autochthonous chicken breeds in the world are already extinct. Among the initial 53 Italian chicken breeds, 70% of them are currently extinct and around 20% are now at risk of extinction [6]. At present, Italian indigenous chicken breeds are neither assured nor protected, and for this reason the country's poultry heritage is becoming increasingly poorer [6].

Different conservation projects have been carried out in Italy over recent years aimed at protecting its native chicken breeds, for example, the "Preserving Biodiversity Project" (BIONET) in the Veneto region [7]; the nation-wide project "Conservation of Biodiversity in Italian Poultry Breeds" (TuBAvi) [8], dedicated to the safeguarding, conservation and improvement of Italian poultry genetic resources; and the project "Germplasm and Agrobiodiversity in Piedmont" (GERMONTE), which employs interventions aimed at characterizing, conserving, and selecting the germplasm of native animal breeds, as well as developing management and breeding plans [9].

A national Italian registry involving 22 native chicken breeds was created and breed standards approved as part of a large cross-sectional conservation project being conducted by the Italian Ministry of Agricultural, Food and Forestry Policies, associated with Ministerial Decree No. 1936 of 1 October 2014 [10].

In recent research carried out by Castillo et al. [6], a questionnaire was administered to Italian breeders aimed at identifying which local breeds were presently being reared across the different Italian regions and to assess the flock numbers of those populations. The two most common native breeds reared were found to be the *Bionda Piemontese* (BP) and the *Livorno* egg-laying hen. On the one hand, some breeds, such as *Collo Nudo Italiano*, *Millefiori Piemontese*, *Pollo Trentino,* and the *Tirolese* chicken, were not listed by breeders at all. Other breeds (*Bianca di Saluzzo* [BS], *Cornuta di Sicilia,* and *Modenese*) were only found to be reared by a small minority of breeders; in fact, only about 20 birds were found for each of these last two breeds. On the other hand, an improving situation was revealed for some breeds that had been studied and that had been the subject of previous conservation projects, such as *Polverara* [6].

From a commercial point of view, local poultry breeds are receiving increasing amounts of consumer interest and represent a smart choice from the biodiversity perspective. Moreover, the use of native chicken breeds has become widely appreciated by consumers in various Western countries, such as France, the UK, the Netherlands, and Germany, in turn, helping to promote genetic variability conservation [11].

Various studies have investigated the meat properties and breed characteristics of Italian chicken breeds with the objective of generating economic interest in these genotypes, obtaining high-quality products for consumers, and promoting genetic biodiversity conservation [5,12–15].

The BP and the BS breeds are the two local chicken breeds originating from the Piedmont region (Northwest Italy) [16]. The BP breed is characterized by its blond plumage and black tail, while the BS breed is completely white. Nowadays, BP and BS breeds are mainly reared for meat production, although they were formerly considered as dual-purpose breeds (for both egg and meat production) [17]. Usually, BP and BS chickens are slaughtered at approximately 180 days of age since it is the first profitable slaughter period for the farmers. However, in some parts of the Piedmont region, these animals are traditionally reared as seasonal products (consumed mainly during Christmas time), and for this kind of product the rearing period could be longer (up to 8 months). Since 2014, these two poultry breeds have been included in a conservation and genetic improvement program run by the University of Turin and they have also been featured in the Slow Food presidium in 2017, a foundation that sustains quality and endangered productions [17]. The BP and the BS possess behavioral and adaptability traits suitable for organic and free-range rearing systems, as sustained by Ferrante et al. [18] and Soglia et al. [17]. Knowledge about the slaughter performance and meat quality of these two local poultry breeds is lacking, but could prove fundamental for promoting their use and, in turn, their conservation. Moreover, no studies on the slaughter performance and meat quality have been provided for the BS and BP breeds regarding differences among sexes.

The aim of the present study was to characterize slaughter performances and meat quality parameters of BP and BS chickens. By providing data on slaughter performances of these two local chickens breeds (both for male and female), the authors would like to provide important information that can improve the rearing of these genotypes, with an indirect positive effect on the genetic conservation of these Italian breeds. Carcass characteristics, proximate composition, and the fatty acid profile of breast and thigh meat of BP and BS chickens were characterized and compared; moreover, the possible effects of breed, sex, and their interaction were evaluated as a source of variability for the considered traits. These parameters were evaluated at different slaughter ages with the objective of providing information for breeders interested in these two genotypes and to identify a market niche for the products obtained.

## 2. Materials and Methods

The study was carried out at the Avian Conservation Centre for Local Genetic Resources of the University of Turin (Italy) located in Carmagnola (TO), Italy. The Avian Conservation Centre was officially awarded in 2016 by the Italian Ministry of Agriculture and Forestry Policies. The trial was approved by the Bioethical Committee of the University of Turin (Italy) (reference no. 451944, 8 November 2019).

### *2.1. Birds and Husbandry*

A total of 320 one-day-old, unsexed chicks from both BS and BP breeds were weighed (average weight 39 g), labelled with a metal wing tag, and reared in indoor pens (2.0 m $\times$ 1.0 m, 40 birds/pen, 4 pens/breed) for up to 8 weeks. Birds were kept in a thermoneutral zone and exposed to a 16:8 (light/dark hours) lighting program. At 8 weeks of age, the sex of each bird was identified by direct visual examination. Then, the chicks were separated according to sex, selected on the base of average live weight (LW), and randomly distributed between 16 pens (2.2 m $\times$ 3.5 m, 15 birds/pen, 4 pens/sex/breed). From 42 to 240 days of age, all birds had access to outdoor runs (2.2 m $\times$ 4.5 m). A total of 240 birds was considered. The pens were equipped with feeders, drinkers, and a suitable shelter to confine chickens at night or during bad weather. From 8 weeks of age onwards, the natural photoperiod was applied (from June, 15:9 light/dark and from November, 9:15 light/dark). All chicks were vaccinated against Marek and Newcastle disease. Chickens had free access to drinking clean and fresh water at all times and were fed ad libitum with a standard commercial starter diet (1–42 days of age) (200 g/kg crude protein, 11.80 MJ metabolizable energy/kg) followed by a growing diet (43–240 days of age) (185 g/kg crude protein, 12.20 MJ metabolizable energy/kg). Clinical signs of illness and mortality were monitored daily throughout the experimental period.

### 2.2. Slaughtering Procedures

Eight males and eight females of each breed and at each slaughter age (5, 6, 7, and 8 months), i.e., 32 birds per age, for an overall total of 128 birds, were selected according to the average final LW in each pen. Each bird was labelled by applying a leg ring and weighed to determine their slaughter weight (SW). The animals were electrically stunned and slaughtered at a commercial abattoir according to standard EU regulations. The plucked and eviscerated carcasses were obtained, and the head, neck, feet, and all abdominal fat removed. Carcasses were stored for 24 h at +4 °C, and then, the chilled carcass (CC) weight was recorded. The CC yield was calculated as a percentage of the SW. Then, the breasts and thighs were excised, and their weights expressed as percentages of the CC weight. A total of 128 breasts and thighs were divided according to the right and left sides, individually vacuum sealed, and refrigerated (4 ± 1 °C). Meat quality parameters (pH 24, color, and drip losses) were assessed on the pectoralis major muscle on the right breast and on the biceps femoris muscle on the right thigh, while the left breast and thigh muscle were freeze-dried and stored at −20 °C until chemical analysis (proximate composition and FA profile).

### 2.3. Meat Quality Parameters

#### 2.3.1. pH

At 24 h post mortem, the pH of the pectoralis major and biceps femoris muscles (under skin, dorsal side) was measured in duplicate using a Crison portable pH meter (Crison Instruments, S.A., Alella, Spain) fitted with a spear-type electrode and an automatic temperature compensation probe.

#### 2.3.2. Color

Breast (pectoralis major, under skin, dorsal side) meat color was measured at 24 h post mortem, using a portable colorimeter Chroma Meter CR-400 Minolta (Minolta Sensing Inc., Osaka, Japan) with an 8 mm diameter measuring area, D65 illuminant, and 2° standard observer. The results were expressed in terms of lightness (L*), redness (a*), and yellowness (b*) in the CIELAB color space (Commission Internationale de l'Éclairage) [19]. Color values were obtained considering the average of three readings per sample.

#### 2.3.3. Drip Losses

At 24 h post mortem, a sample of breast meat was weighed and placed within a container on a supporting mesh and sealed. The samples were blotted for excess surface fluids and re-weighed. Drip losses were determined as percentage of weight lost by the samples during the refrigerated storage period (24 h, at 4 ± 1 °C) [20].

#### 2.3.4. Proximate Composition

Breast and thigh samples were cut, homogenized, and divided into two parts. One portion was used to determine moisture (#950.46) and ash (#920.153) contents according to AOAC International procedures [21]. The remaining part was freeze-dried and analyzed for crude protein (CP) and crude fat (CF) contents, and fatty acid (FA) composition.

In order to estimate the meat CP content, the total N amount was determined, according to the Dumas method [22], using a Macro-N Nitrogen Analyzer (Foss Heraeus Analysensysteme, Hanau, Germany). The CP content was calculated by multiplying the measured nitrogen quantity by the appropriate nitrogen-to-protein conversion factor (6.25). The CF content was determined by Soxhlet extraction with petroleum ether, according to AOAC International method #991.36 [21]. Proximate composition results are expressed as g/100 g of fresh matter (FM).

#### 2.3.5. Hydroxyproline

The collagen content in breast muscle samples was measured indirectly using the method for hydroxyproline (HP) analysis developed by Reddy and Enwemeka [23]. Briefly, 30–50 mg of muscle samples was frozen in liquid nitrogen, and then, lyophilized. The assay employed 2 mL capacity O'-ring screw-capped Nalgene high temperature polypropylene tubes. Aliquots of standard HP or test samples were hydrolyzed in sodium hydroxide (2 N final concentration). Then, the hydrolyzed tissues were mixed with a buffered chloramine-T reagent, and the oxidation was allowed to proceed for 25 min at room temperature. The chromophore was, then, developed by the addition of Ehrlich's reagent, and the absorbance of the reddish-purple complex was measured at 550 nm using a spectrophotometer. Absorbance values were plotted against the concentration of standard hydroxyproline, and the presence of HP in unknown tissue extracts was determined from the standard curve. HP content is expressed as mg/g dry weight.

#### 2.3.6. Fatty Acid Profile

The FA profile was determined following the method reported by Glass and Cristopherson [24]. Briefly, 250 μg of lipids and 500 μL of a solution of KOH in methanol 2 N were put into a vial containing 5 mL of hexane and 1 g of anhydrous sodium sulphate. The vial was mixed for 30 s and placed in a water bath at 40 °C for 15 min. The sample was cooled on ice after had been stirred. The fatty acid methyl esters (FAME), contained in the upper phase, were collected. Then, they were separated, identified, and quantified using a Shimadzu GC17A gas chromatograph (Shimadzu Corporation, Tokyo, Japan) with a WP-4 Shimadzu integration system equipped with a Varian CPSIL88 capillary column (100 m long, 0.25 mm i.d., 0.20 mm film thickness) (Varian, Walnut Creek, CA, USA) and a flame ionization detector. The gas chromatograph operated in the following conditions: the oven temperature was kept at 170 °C for 15 min, reached 190 °C at a rate of 1 °C/min, then to 220 °C at a rate of 5 °C/min, and kept at this temperature for 17 min. The temperatures of the injector and detector were maintained at 270 °C and 300 °C, respectively. Helium was used as the carrier gas at a constant flow rate of 1.7 mL/min. The individual FAME were identified by comparison with commercial standards (PUFA-2 fatty acid methyl ester standards, Matreya, Pleasant Gap, PA, USA). Quantification was achieved using methyl nonadecanoate 98% (C19:0) (Sigma, Saint Louis, MO, USA) as the internal standard, which was added prior to lipid extraction. The results are expressed as a percentage of each individual FAME per total FAME detected.

### *2.4. Statistical Analysis*

The statistical analysis was performed using IBM SPSS Statistics v.27.0 for Windows (IBM SPSS Statistics, Armonk, NY, USA). The effects of breed and gender and their interaction, at each of the four consecutive slaughter ages, on the carcass characteristics, as well as on quality parameters, proximate composition, and FA profile of meat, were analyzed using the following General Linear Model of fixed effects:

$$Y_{ijk} = \mu + Breed_i + Sex_j + Breed \times Sex_{ij} + e_{ijk}$$

where $Y_{ij}$ is the dependent variable, μ is the overall mean, $Breed_i$ is the breed effect (i = 1–2), $Sex_j$ is the sex effects (j = male and female), Breed × $Sex_{ij}$ is the interaction effect between Breed and Sex, and $e_{ijk}$ is the observational error.

Moreover, a further statistical analyses was performed, adding age at slaughter as a fixed component to the previous model; therefore, when this effect was significant ($p < 0.05$), the Duncan procedure as post hoc test was applied to investigate any differences between the different slaughter ages. The assumption of normality and homogeneity of variance was assessed using Shapiro–Wilk and Levene's tests, respectively. The results are reported as means plus the standard error of the mean (SEM). Significance is declared for $p < 0.05$.

## 3. Results

With the exception of Table 1, the data reported in the text below represent the average results for the four slaughter ages. Those for the separate slaughter ages are instead reported in the Supplementary Materials.

**Table 1.** Carcass weights of *Bionda Piemontese* and *Bianca di Saluzzo* breeds at different slaughter ages ($n = 8$).

| Items | Age | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-Value | |
|---|---|---|---|---|---|---|---|---|
| | | **Male** | **Female** | **Male** | **Female** | | **Breed** | **Gender** |
| SW (g) | 5 months | 1915.00 $^{c}$ | 1505.40 $^{c}$ | 1857.46 $^{d}$ | 1545.08 $^{b}$ | 37.469 | 0.818 | <0.001 |
| | 6 months | 2187.48 $^{b}$ | 1706.58 $^{b}$ | 2260.53 $^{c}$ | 1695.13 $^{ab}$ | 49.017 | 0.276 | <0.001 |
| | 7 months | 2433.38 $^{a}$ | 1808.63 $^{ab}$ | 2471.25 $^{b}$ | 1928.88 $^{a}$ | 65.887 | 0.345 | <0.001 |
| | 8 months | 2601.89 $^{a}$ | 1884.78 $^{a}$ | 2649.33 $^{a}$ | 1859.30 $^{a}$ | 76.416 | 0.884 | <0.001 |
| *p*-Value | | <0.001 | <0.001 | <0.001 | 0.019 | | | |
| CC weight (g) | 5 months | 1148.95 $^{c}$ | 910.32 $^{b}$ | 1075.70 $^{c}$ | 912.24 | 22.548 | 0.150 | <0.001 |
| | 6 months | 1325.38 $^{b}$ | 1012.50 $^{ab}$ | 1342.38 $^{b}$ | 978.13 | 33.044 | 0.748 | <0.001 |
| | 7 months | 1497.94 $^{a}$ | 1042.47 $^{a}$ | 1464.27 $^{a}$ | 1032.02 | 44.209 | 0.585 | <0.001 |
| | 8 months | 1528.00 $^{a}$ | 1072.50 $^{a}$ | 1516.00 $^{a}$ | 980.13 | 49.622 | 0.250 | <0.001 |
| *p*-Value | | <0.001 | 0.015 | <0.001 | 0.285 | | | |

Abbreviations: SW, slaughter weight; CC, chilled carcass; SEM, standard error of the mean. Values with different superscript letters (a–c) within the same column per fixed effect (age of slaughter) differ significantly ($p < 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

### 3.1. Slaughter Traits

Table 1 reports the SW and CC weight of BP and BS breeds at each of the four different slaughter ages. In general, a significant effect of gender on the SW and CC weight was observed at each slaughter age ($p < 0.001$). Slaughter age also had a significant effect on SW and CC weight, with the highest values recorded at 7 and 8 months of age ($p < 0.05$), except for BS males, for whom a significant difference in SW was also observed between the two older slaughter ages (being higher at 8 months than at 7 months of age) ($p < 0.05$). Moreover, in BS female birds, the CC weight was unaffected by slaughter age.

The average slaughter traits and breast and thigh yields of the BP and BS chicken breeds are reported in Table 2. As reported above, gender significantly affected the SW and CC weight of the birds ($p < 0.001$). Moreover, the carcass, breast, and thigh yields were affected by gender ($p < 0.001$). Chicken breed did have a significant effect on carcass yield (the lowest value was observed for BS female birds, with an average value of 55.88%, $p < 0.001$).

**Table 2.** Average slaughter yields for *Bionda Piemontese* and *Bianca di Saluzzo* breeds. Data are the average of the four consecutive slaughterings at 5, 6, 7, and 8 months of age ($n = 32$).

| Items | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-Value | |
|---|---|---|---|---|---|---|---|
| | **Male** | **Female** | **Male** | **Female** | | **Breed** | **Gender** |
| SW (g) | 2284.43 | 1726.34 | 2309.64 | 1757.09 | 34.59 | 0.570 | <0.001 |
| CC weight (g) | 1375.07 | 1009.44 | 1349.58 | 975.63 | 21.24 | 0.277 | <0.001 |
| Carcass yield (%SW) | 60.28 | 58.53 | 58.44 | 55.88 | 0.314 | <0.001 | <0.001 |
| Breast yield (%CC weight) | 16.80 | 19.10 | 17.29 | 19.04 | 0.172 | 0.455 | <0.001 |
| Thigh yield (%CC weight) | 39.09 | 32.57 | 38.96 | 32.22 | 0.697 | 0.383 | <0.001 |

Abbreviations: SW, slaughter weight; CC, chilled carcass; SEM, standard error of the mean. The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

Specifically, at 8 months of age, as compared with female birds, the males of both breeds showed greater SW (2625.61 $\pm$ 33.545 g, average data for the two breeds), CC weights (1522.00 $\pm$ 8.485 g), and thigh yields (39.95%) (the average values for females were: 1872.04 $\pm$ 18.017 g, 1026.31 $\pm$ 65.315 g and 32.40%, respectively, $p < 0.05$) (Table S1).

Moreover, the considered breed significantly affected average carcass yields at 5 (60.25 vs. 58.49% SW for BP and BS, respectively), 7 (59.56 vs. 56.70% SW for BP and BS, respectively), and 8 months of age (57.85 vs. 54.97% SW for BP and BS, respectively, Table S1). Slaughter ages significantly affected carcass yields of BS females, with higher percentages produced at 5 and 6 months of age ($p < 0.05$) (Table S1). However, this parameter did not affect the thigh yield of the two chicken breeds ($p > 0.05$).

Contrary to that observed for the thigh yields, the females of both breeds produced larger breast yields than males (on average 10.62% higher than males, $p < 0.001$) (Table 2). Breast yield was also influenced by the slaughter age of the two chicken breeds, showing the highest values at 5, 7, and 8 months of age for BP female and for BS male and female birds ($p < 0.05$) (Table S1).

### 3.2. Meat Quality Parameters

The average pH (at 24 h from slaughtering), color, and drip loss values for breast and thigh meat are reported in Table 3. Significant differences were observed between the two breeds for the yellowness (b*) of meat, which resulted higher in BP as compared with BS (average values 9.50 vs. 8.72, respectively, $p < 0.05$). Moreover, gender significantly influenced all the evaluated parameters ($p < 0.05$), except for the pH of thigh meat and breast drip losses, which were instead similar between the sexes ($p > 0.05$) (Table 3).

**Table 3.** Average meat quality parameters for breast and thigh of *Bionda Piemontese* and *Bianca di Saluzzo* breeds. Data are the average of the four consecutive slaughterings at 5, 6, 7, and 8 months of age ($n = 32$).

| Items | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-Value | |
|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | | Breed | Gender |
| pH breast | 5.91 | 5.81 | 5.88 | 5.84 | 0.014 | 0.995 | 0.010 |
| pH thigh | 6.20 | 6.26 | 6.26 | 6.23 | 0.016 | 0.533 | 0.704 |
| Breast L* | 51.22 | 53.90 | 51.13 | 54.12 | 0.271 | 0.884 | <0.001 |
| Breast a* | 1.89 | −0.05 | 1.40 | −0.61 | 0.190 | 0.123 | <0.001 |
| Breast b* | 8.62 | 10.39 | 8.10 | 9.35 | 0.201 | 0.041 | <0.001 |
| Breast drip loss (%) | 8.12 | 6.56 | 7.46 | 6.57 | 0.331 | 0.625 | 0.067 |

Abbreviations: L*, lightness; a*, redness; b*, yellowness; SEM, standard error of the mean. The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

In more detail, gender had little effect on meat pH, with differences only detected at 6 months of age for breast pH and at 5 months of age for thigh pH ($p < 0.05$) (Table S2). The thigh meat pH was significantly affected by the age of female birds (thigh pH in BP females was highest at 7 months of age, whereas in BS females the highest thigh pH values were observed at 6 and 8 months of age, $p < 0.05$), whereas the results in terms of breast meat pH showed no differences at the different slaughter ages, except in BS females (Table S2).

In general, breast lightness (L*), redness (a*), and yellowness (b*) were mainly affected by gender. Higher levels of lightness (L*) and yellowness (b*) were observed in the females of both BP and BS birds, and higher values for redness (a*) were detected in BP and BS males ($p < 0.001$) (Table 3). Specifically, breast lightness (L*) was influenced by gender at 6 and 8 months of age, with higher values in females than males in both the considered breeds (Table S2). Regarding the different slaughter ages, significant differences in breast lightness (L*) were only evident in BS males, with the lowest value observed in birds aged 8 months ($p < 0.001$) (Table S2).

Breast redness (a*) was significantly affected by both gender and slaughter age ($p < 0.001$). Indeed, the females of both BP and BS breeds presented lower redness (a*) levels in breast meat than males at 5, 7, and 8 months of age ($p < 0.001$). Slaughter age had a significant effect on all the birds, with the highest values observed at 8 months of age for this parameter ($p < 0.05$) (Table S2).

Breast yellowness (b*) changed in relation to gender and breed at 6 months of age. Indeed, at 6 months of age, BP showed higher yellowness (b*) values than BS (9.5 vs. 7.99, respectively), and females of both BP and BS showed higher values than males (10.38 vs. 7.12, respectively) (Table S2).

Finally, the average drip loss values were similar between the two breeds and sexes (Table 3). However, when taking the different slaughter ages into consideration, drip losses were significantly influenced by gender at 7 and 8 months of age ($p < 0.05$) (Table S2). Indeed, average drip losses in males were 21.08% greater than in females at 8 months of age ($p < 0.05$) (Table S2). Drip losses were influenced by slaughter age, steadily decreasing as the animals became older ($p < 0.001$) (Table S2).

### 3.3. Breast Meat Chemical Composition and Hydroxyproline Content

The average chemical composition values (moisture, CP, CF, and ash) and hydroxyproline contents of breast meat are summarized in Table 4. Moisture, as well as CF and CP contents were affected by gender ($p < 0.001$). Moisture and CP content were higher in males than in females, whereas the CF content was higher in females than in males ($p < 0.05$) (Table 4).

**Table 4.** Average chemical composition (g/100 g FM) and hydroxyproline content (mg/g dry weight) of breast meat of *Bionda Piemontese* and *Bianca di Saluzzo* breeds. Data are the average of the four consecutive slaughtering at 5, 6, 7, and 8 months of age ($n$ = 32).

| Items | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-Value | |
|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | | Breed | Gender |
| Moisture | 74.35 | 73.32 | 74.16 | 73.42 | 0.088 | 0.789 | <0.001 |
| Crude protein | 25.00 | 24.73 | 25.61 | 24.84 | 0.113 | 0.105 | 0.021 |
| Crude fat | 0.31 | 0.89 | 0.22 | 0.75 | 0.048 | 0.177 | <0.001 |
| Ash | 1.17 | 1.20 | 1.17 | 1.18 | 0.005 | 0.308 | 0.062 |
| Hydroxyproline | 2.291 | 2.548 | 2.337 | 2.706 | 0.198 | 0.607 | 0.118 |

Abbreviations: FM, fresh matter; SEM, standard error of the mean. The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

In more detail, breed did not generally affect the chemical composition of breast meat, except for CF and ash content (at 8 and 6 months of age, respectively, $p < 0.05$) (Table S3). Gender significantly affected moisture (except at 8 months of age) and CF content ($p < 0.05$) (Table S3). At 8 months of age, gender is responsible for a statistical trend for CF content, with the highest values being observed in female birds of the two chicken breeds ($p = 0.056$).

Males showed higher breast moisture and lower CF contents than females (Table S3). Moreover, the CP and ash contents of breast meat were influenced by gender at 5 months of age only (highest CP values in males, lowest ash value in females, $p < 0.05$) (Table S3). Slaughter age mainly affected the moisture and CF contents of breast meat. Higher values of moisture were observed at 5 and 6 months of age for BS and BP males and BP females, respectively. The highest CF value in BP and BS females was observed at 7 months of age. However, in BS males, the highest value was observed at 6, 7, and 8 months of age ($p < 0.05$) (Table S3). Ash content in BS males was also affected by the slaughter age, with the highest values found at 7 and 8 months of age ($p < 0.05$).

Gender affected HP content at 5 months of age (Table S3). Age of slaughter affected HP content in BP females and BS males, with the highest values being observed at 7 and 8 months of age in BS males, and at 5, 6, and 7 months of age in BP females (Table S3).

### 3.4. Thigh Meat Chemical Composition

In general, a significant effect of chicken breed was observed in relation to thigh CP content (being higher in BS than BP), whereas gender significantly affected CP, CF, and ash contents ($p < 0.05$) (Table 5). Indeed, the females showed higher CP and CF contents than males, while lower ash levels were observed in females than in males ($p < 0.05$) (Table 5).

**Table 5.** Average chemical composition (g/100 g FM) of thigh meat of *Bionda Piemontese* and *Bianca di Saluzzo* breeds. Data are the average of the four-consecutive slaughtering at 5, 6, 7, and 8 months of age ($n = 32$).

| Items | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-Value | |
|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | | Breed | Gender |
| Moisture | 75.29 | 74.93 | 75.41 | 74.64 | 0.304 | 0.893 | 0.357 |
| Crude protein | 27.22 | 27.53 | 27.50 | 28.82 | 0.181 | 0.027 | 0.022 |
| Crude fat | 1.71 | 4.46 | 1.79 | 4.34 | 0.168 | 0.934 | <0.001 |
| Ash | 1.11 | 1.00 | 1.09 | 1.00 | 0.012 | 0.734 | <0.001 |

Abbreviations: FM, fresh matter; SEM, standard error of the mean. The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

In more detail, gender significantly affected moisture and CF content ($p < 0.05$) (Table S4). Females showed a higher moisture content (+6.80%) than males at 5 months of age. By contrast, moisture content was higher in males than in females at 6, 7, and 8 months of age ($p < 0.001$) (Table S4). Females of both chicken breeds showed higher CF contents at all slaughter ages ($p < 0.001$) (Table S4). The CP content was affected by gender at 6 months of age only, with higher CP levels seen in males than in females.

Finally, regarding the ash content, males had significantly higher levels than females at 5 and 7 months of age (1.08 vs. 0.76 and 1.09 vs. 1.02 g/100 g FM, respectively).

The chemical composition of thigh meat was significantly influenced by slaughter age, since moisture content was significantly higher at 5 months of age as compared with the other slaughter ages in both breeds ($p < 0.05$) (Table S4). On the contrary, the lowest values of thigh CF content were observed at 5 months of age in both chicken breeds ($p < 0.05$) (Table S4).

### 3.5. Fatty Acid Profile of Breast Meat

The overall saturated fatty acid (SFA) composition of breast meat was mainly influenced by gender ($p < 0.05$) (Table 6). In more detail, gender had a significant effect on the total SFA content at 6 and 8 months of age, with male birds showing higher concentrations ($p < 0.05$) (Table S5). Breed affected total SFA content at 7 months of age only, with BP birds showing greater overall SFA levels than BS birds ($p < 0.05$) (Table S5). Slaughter age also affected the total SFA content of breast meat, except for the BS females ($p < 0.05$) (Table S5). SFA levels in BP males increased from 5 to 7 months of age, while no differences were recorded between 7 and 8 months of age. By contrast, the breast SFA content in BS males was higher at 8 months of age as compared with the other slaughter ages ($p < 0.05$) (Table S5). Finally, in BP females, the highest SFA levels in breast meat were observed at 7 months of age ($p < 0.01$) (Table S5).

The averaged data show palmitic acid (C16:0) to be the most abundant SFA in breast meat, the levels of which varied according to breed (being higher in BP than in BS birds) ($p < 0.05$) (Table 6). More specifically, palmitic acid content was influenced by breed at 7 months of age, with the highest value recorded in the BP genotype ($p < 0.05$) (Table S5). At each slaughter age, gender also affected breast palmitic acid content, with females showing higher levels than males ($p < 0.05$) (Table S5). In BP birds of both genders, the palmitic acid content changed in relation to slaughter age, with the lowest values observed at 5 and 6 months ($p < 0.05$) (Table S5).

**Table 6.** Average fatty acid profile for breast meat (g/100 g of total detected fatty acids) of *Bionda Piemotese* and *Bianca di Saluzzo* breeds. Data are the average of the four-consecutive slaughtering at 5, 6, 7, and 8 months of age ($n$ = 32).

| Items | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-Value | |
|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | | Breed | Gender |
| C14:0 | 0.42 | 0.64 | 0.42 | 0.51 | 0.052 | 0.533 | 0.128 |
| C16:0 | 28.14 | 29.41 | 26.94 | 27.76 | 0.294 | 0.014 | 0.072 |
| C17:0 | 0.34 | 0.31 | 0.24 | 0.31 | 0.020 | 0.239 | 0.543 |
| C18:0 | 14.23 | 11.61 | 14.73 | 12.03 | 0.172 | 0.071 | <0.001 |
| C24:0 | 1.07 | 1.03 | 1.46 | 0.90 | 0.058 | 0.263 | 0.009 |
| ΣSFA | 44.20 | 43.01 | 43.78 | 41.52 | 0.349 | 0.165 | 0.012 |
| C16:1n7 | 1.20 | 2.34 | 0.90 | 2.09 | 0.091 | 0.062 | <0.001 |
| C18:1c9 | 28.52 | 35.00 | 28.18 | 34.31 | 0.498 | 0.535 | <0.001 |
| C20:1 | 0.27 | 0.20 | 0.15 | 0.27 | 0.017 | 0.520 | 0.423 |
| C24:1 | 0.42 | 0.50 | 0.69 | 0.50 | 0.038 | 0.074 | 0.503 |
| ΣMUFA | 30.41 | 38.05 | 29.91 | 37.18 | 0.563 | 0.459 | <0.001 |
| C18:2n6 | 13.38 | 10.91 | 13.13 | 11.48 | 0.313 | 0.791 | 0.001 |
| C20:2n6 | 0.26 | 0.14 | 0.29 | 0.17 | 0.017 | 0.402 | <0.001 |
| C20:4n6 | 6.35 | 4.08 | 7.05 | 5.19 | 0.370 | 0.213 | 0.005 |
| Σn6 PUFA | 19.99 | 15.14 | 20.47 | 16.88 | 0.596 | 0.333 | <0.001 |
| C18:3n3 | 0.08 | 0.21 | 0.05 | 0.17 | 0.017 | 0.192 | <0.001 |
| C20:5n3 | 0.45 | 0.23 | 0.70 | 0.33 | 0.078 | 0.257 | 0.059 |
| C22:5n3 | 0.61 | 0.37 | 0.93 | 0.47 | 0.043 | 0.009 | <0.001 |
| C22:6n3 | 0.55 | 0.64 | 0.82 | 0.68 | 0.050 | 0.141 | 0.812 |
| Σn3 PUFA | 1.70 | 1.47 | 2.50 | 1.65 | 0.130 | 0.054 | 0.033 |
| ΣPUFA | 21.69 | 16.60 | 22.97 | 18.49 | 0.668 | 0.217 | <0.001 |
| Other FA | 3.31 | 2.21 | 2.89 | 2.60 | 0.157 | 0.969 | 0.026 |

Abbreviations: ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; FA, fatty acids; SEM, standard error of the mean. The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

Stearic acid (C18:0) was the second most abundant FA in breast meat, mainly being affected by gender (higher in males) (Table 6). Specifically, a significant effect of gender on stearic acid content was observed at 6, 7, and 8 months of age. Breed significantly affected breast meat stearic acid content at 7 and 8 months of age, with opposite patterns being observed in BP vs. BS. Indeed, at 7 months of age BP birds showed higher levels of stearic acid than BS (+5.86%, on average), whereas at 8 months of age the opposite was true, with BS birds showing higher levels than BP birds (+9.8%, $p < 0.05$) (Table S5).

The monounsaturated fatty acid (MUFA) content of breast meat was mainly affected by gender, being higher in female birds ($p < 0.001$) (Table 6). Breed had little impact, with BP birds showing a higher MUFA content than BS birds at 8 months of age only ($p < 0.05$) (Table S6). Slaughter age was associated with significant differences. MUFA content in the females of both breeds increased significantly from 6 to 7 months of age (+23.34% and +21.11%, for BP and BS, respectively), whereas it decreased significantly from 7 to 8 months of age ($p < 0.05$) (Table S6). BS male birds showed a lower MUFA content in breast meat at 5 months as compared with all other slaughter ages ($p < 0.001$), whereas no significant evidence was recorded in the BP male group (Table S6).

Oleic acid (C18:1c9) was the most abundant MUFA in breast meat, and was generally higher in female birds as compared with males ($p < 0.001$) (Table 6). Specifically, gender affected breast meat oleic acid levels at 5, 7, and 8 months of age ($p < 0.001$), whereas breed had little effect on this FA, only having an effect at 8 months of age, when it was higher in BP birds as compared with BS birds ($p < 0.05$) (Table S6). Slaughter age was associated with significant differences in oleic acid content. Similar patterns were observed among

BP and BS males, which showed the highest oleic acid concentrations at 8 and 7 months of age, respectively ($p < 0.05$) (Table S6). In addition, both BP and BS females exhibited a peak oleic acid content at 7 months of age ($p < 0.001$) (Table S6).

Overall, the Σn6 PUFA content was higher in male than in female birds ($p < 0.001$) (Table 6). In more detail, gender affected breast meat Σn6 PUFA at 5 and 7 months of age, with males showing higher Σn6 PUFA levels than females ($p < 0.05$) (Table S7). The Σn6 PUFA content was significantly higher at 5 months of age as compared with all other slaughter ages ($p < 0.001$) (Table S7).

Linoleic acid (C18:2n6) was the most abundant n6 PUFA in breast meat. It was significantly affected by gender, with the highest levels being observed in males ($p < 0.05$) (Table 6). This FA showed significant differences at 7 months of age, being affected by both breed and gender ($p < 0.05$) (Table S7). Higher linoleic acid levels were observed in male birds of both breeds, although the breast meat of BP birds contained less linoleic acid than in BS birds ($p < 0.001$) (Table S7). A different picture was evident at 6 months of age when BP birds showed higher linoleic acid levels as compared with BS birds ($p < 0.05$) (Table S7). Slaughter age also affected the linoleic acid content of female BP birds, with lower levels recorded at 6 months of age ($p < 0.001$).

Similarly, the average data related to arachidonic acid (C20:4n6) showed differences between the sexes, being higher in males than in females ($p < 0.05$) (Table 6). Moreover, significant differences were also evident among males and females and between the two genders at 5 months of age ($p < 0.05$) (Table S7). Specifically, the males contained more arachidonic acid than the females, and the BP genotype had lower levels as compared with the BS genotype ($p < 0.05$) (Table S7). The breast meat arachidonic acid levels were highest at 5 months of age in all the birds, but had significantly decreased by the time animals were slaughtered at 8 months of age ($p < 0.001$) (Table S7).

The overall results for Σn3 PUFA showed higher levels of these FA in male than in female birds ($p < 0.05$) (Table 6). In particular, the males showed a higher Σn3 PUFA content than females at 5 and 7 months of age ($p < 0.05$) (Table S7). Moreover, the Σn3 PUFA levels were higher in BS birds as compared with BP birds at 7 months of age ($p < 0.05$) (Table S7). Slaughter age significantly affected Σn3 PUFA in both genders of the BP genotype, and BP female birds ($p < 0.05$) (Table S7).

The specific n3 PUFAs considered were: linolenic (C18:3n3), eicosapentaenoic (C20:5n3), docosapentaenoic (C22:5n3), and docosahexaenoic (C22:6n3) acid, each of which was detected in very small amounts in breast meat (Table 6 and Table S7).

In general, the PUFA contents of breast meat were higher in male than in female birds ($p < 0.001$) (Table 6). Considering the data at the different slaughter ages this pattern was confirmed at 5 and 7 months of age ($p < 0.05$) (Table S7). Moreover, the breast meat of BP birds contained less PUFA than the BS group at 7 months of age ($p < 0.05$) (Table S7), and PUFA levels decreased as the birds aged ($p < 0.05$) (Table S7).

### 3.6. Fatty Acid Profile of Thigh Meat

In Table 7, the thigh meat FA profiles of the two breeds of chicken are summarized, as well as the average values of the different slaughter ages considered. The overall total SFA content of thigh meat was affected by both breed and gender. Indeed, a higher SFA amount was detected in BP than BS, and males showed more SFA than females ($p < 0.001$) (Table 7). Breed and the gender both affected the thigh SFA content at 7 and 8 months of age, and the BP thigh meat contained more SFA than the BS genotype ($p < 0.05$) (Table S8).

**Table 7.** Average fatty acid profile of thigh meat (g/100 g of total detected fatty acids) of *Bionda Piemontese* and *Bianca di Saluzzo* breeds. Data are the average of the four consecutive slaughtering at 5, 6, 7, and 8 months of age ($n = 32$).

| Items | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-Value | |
|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | | Breed | Gender |
| C14:0 | 0.89 | 1.13 | 0.77 | 1.00 | 0.019 | <0.001 | <0.001 |
| C16:0 | 32.60 | 36.24 | 30.79 | 34.64 | 0.254 | <0.001 | <0.001 |
| C17:0 | 0.45 | 0.43 | 0.46 | 0.41 | 0.010 | 0.810 | 0.074 |
| C18:0 | 20.07 | 12.60 | 19.98 | 13.24 | 0.371 | 0.497 | <0.001 |
| C24:0 | 0.56 | 1.07 | 0.64 | 0.88 | 0.041 | 0.460 | <0.001 |
| ΣSFA | 54.57 | 51.47 | 52.63 | 50.17 | 0.274 | 0.001 | <0.001 |
| C16:1n7 | 2.67 | 4.50 | 2.58 | 4.18 | 0.103 | 0.138 | <0.001 |
| C18:1c9 | 34.98 | 38.19 | 35.39 | 39.55 | 0.287 | 0.061 | <0.001 |
| C20:1 | 0.40 | 0.35 | 0.49 | 0.39 | 0.015 | 0.025 | 0.010 |
| C24:1 | 0.18 | 0.23 | 0.19 | 0.15 | 0.018 | 0.334 | 0.975 |
| ΣMUFA | 38.24 | 43.26 | 38.65 | 44.28 | 0.363 | 0.202 | <0.001 |
| C18:2n6 | 4.38 | 2.22 | 4.72 | 2.17 | 0.175 | 0.597 | <0.001 |
| C20:2n6 | 0.02 | 0.01 | 0.02 | 0.01 | 0.003 | 0.818 | 0.028 |
| C20:4n6 | 0.51 | 0.21 | 0.76 | 0.46 | 0.064 | 0.049 | 0.017 |
| Σn6 PUFA | 4.91 | 2.43 | 5.50 | 2.64 | 0.195 | 0.204 | <0.001 |
| C18:3n3 | 0.14 | 0.09 | 0.13 | 0.09 | 0.012 | 0.679 | 0.049 |
| C20:5n3 | 0.03 | 0.19 | 0.02 | 0.06 | 0.023 | 0.096 | 0.023 |
| C22:5n3 | 0.00 | 0.03 | 0.16 | 0.04 | 0.026 | 0.105 | 0.349 |
| C22:6n3 | 0.06 | 0.03 | 0.23 | 0.03 | 0.039 | 0.263 | 0.134 |
| Σn3 PUFA | 0.23 | 0.35 | 0.55 | 0.21 | 0.067 | 0.505 | 0.408 |
| ΣPUFA | 5.14 | 2.78 | 6.05 | 2.85 | 0.210 | 0.152 | <0.001 |
| Other FA | 2.04 | 2.48 | 2.66 | 2.71 | 0.129 | 0.105 | 0.343 |

Abbreviations: ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; FA, fatty acids; SEM, standard error of the mean. The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

In general, C16:0 and C18:0 were the most abundant FAs in the thigh meat of both chicken breeds. Breed and gender significantly affected the average palmitic acid (C16:0) content in thigh meat, with BP showing higher levels compared with BS, and higher amount recorded in females than males ($p < 0.05$) (Table 7). Specifically, palmitic acid content was significantly higher in BP birds of 5 and 8 months of age than in BS birds of the same age, and it was always higher in females than in males ($p < 0.001$) (Table S9). Furthermore, a significant incidence effect for the different slaughter ages was only observed in the BS breed, since males showed their highest values at 5, 6, and 7 months of age, whereas in females, the highest values were detected at 6, 7, and 8 months of age ($p < 0.05$) (Table S9).

Gender significantly influenced the average stearic acid (C18:0) levels in thigh meat (being higher in males than in females) ($p < 0.001$) (Table 7). In more detail, gender had a significant incidence on stearic acid content at all slaughter ages, and was always higher in males than in females ($p < 0.001$) (Table S9). Furthermore, only at 5 months of age did breed significantly affect stearic acid content, when levels in the BS genotype were about 11.17% higher than in BP ($p < 0.05$) (Table S9). Slaughter age significantly affected the stearic acid levels in BP males and BS females, with higher levels at 7 and 8 months in BP males, and at 5, 6, and 8 months of age in BS females ($p < 0.05$) (Table S9).

The average MUFA content in thigh meat was higher in females than in males ($p < 0.001$) (Table 7). In more detail, females showed higher MUFA contents than males at all the different slaughter ages ($p < 0.001$) (Table S9). Furthermore, statistical differences were recorded between breeds at 7 months of age, with the BS genotype showing a higher MUFA content than the BP genotype ($p < 0.05$) (Table S9).

Oleic acid (C18:1c9) was the predominant MUFA in thigh meat in all birds, and it was generally affected by the gender of birds, with females showing the highest levels ($p$ <0.001) (Table 7). The effects of gender on oleic acid content were detected at all the slaughter ages studied ($p < 0.05$) (Table S9), whereas breed was only found to have a significant effect on thigh oleic acid content at 7 months of age, being higher in the BS genotype as compared with BP birds ($p < 0.05$) (Table S9). Finally, this FA was also influenced by age of slaughter in the BS male group, with significant increases recorded at 6, 7, and 8 months of age ($p < 0.05$) (Table S9).

In general, Σn6 PUFA content was affected by gender, with higher levels recorded in male birds ($p < 0.001$) (Table 7). The gender effect on Σn6 PUFA content could also be observed at different slaughter ages ($p < 0.05$) (Table S10). Moreover, Σn6 PUFA content was influenced by age of slaughter, except in BP male birds ($p < 0.05$) (Table S10). Females of both breeds showed higher values at 5 months of age, while male birds of the BS genotype were instead characterized by an irregular trend ($p < 0.05$) (Table S10).

The most abundant n6 PUFA detected in thigh meat was linoleic acid (C18:2n6), which was significantly affected, however, by gender, being higher in males than in females ($p < 0.001$) (Table 7). In more detail, linoleic acid content varied in relation to gender independently of slaughter age. The age of slaughter influenced thigh linoleic acid content in only the females of either breed, with significantly higher values observed at 5 months of age than the other months considered ($p < 0.05$) (Table S10).

The Σn3 PUFA were the least abundant fatty acids in thigh meat (Table 7). More specially, the only significant difference was observed in the thigh meat from BS females, which was richest in these fatty acids at 5 months of age ($p < 0.05$) (Table S10).

Gender significantly affected the overall PUFA content ($p < 0.001$) (Table 7), being consistently higher in males as compared with females ($p < 0.05$) (Table S10). Statistical differences were observed considering age of slaughter in BP females and in both sexes of the BS genotype. The BP and BS females showed the highest PUFA content at 5 months of age ($p < 0.05$) (Table S10). The highest PUFA values in BS males were recorded at 5 and 8 months of age ($p < 0.05$) (Table S10).

## 4. Discussion

The SW of all birds in the present work increased according to an increase in slaughter age, in agreement with the data reported for BP and BS breeds by Soglia et al. [17]. The SW of BP and BS breeds at 6 months of age was lower than previously reported for the *Milanino* chicken breed slaughtered at that age (which averaged 2843 g and 2318 g for males and females, respectively) [25]. On the contrary, the recorded SW at 5 months of age were slightly higher in BP and BS as compared with those reported for the *Padovana* breed (1882 g for males and 1328 g for females) [26].

Moreover, the CC weight of all birds in the present work increased according to an increase in slaughter age, whereas carcass yields were not influenced by this parameter. Interestingly, the contrary was observed in the BS females, in which the CC weight did not undergo any significant change between 5 and 8 months of age, while the carcass yield was affected by this variable. However, significant differences in terms of carcass yields were observed in relation to breed, even though breed had no significant effect on either SW or CC weight. The better carcass yields observed for BP birds as compared with BS birds (at 5, 7, and 8 months of age) could be linked to more favorable genetic characteristics that should be investigated in future work. Indeed, Zanetti et al. [5] observed significant differences in accordance with genetic traits, in terms of live and carcass weights, between *Padovana*, *Ermellinata*, and *Pepoi* chicken breeds. The recorded carcass yields in the present study were lower than the previously reported results for other Italian chicken breeds, such as the *Romagnola* (62%) and the *Modenese* (63%) breeds [27].

The sexual dimorphism observed in the present study was in line with the findings recorded in BP and BS breeds by Soglia et al. [17]. Similarly, the body weights of the *Géline de Touraine* genotype presented significant differences between males and females, despite similar carcass yields between the two sexes [28]. The higher carcass yields detected in male birds (of both BP and BS) as compared with females in the present work confirmed this gender difference.

Interestingly, the female birds of the BS genotype showed a better carcass yield at 5 and 6 months of age as compared with 7 and 8 months of age, and this result may be related to a later development of the reproductive system and organs that affected the live weight of animals without increasing the carcass weight.

In the current work, the breast yields varied in relation to the slaughter age and gender of the birds. Females displayed higher breast yields than males, whereas males showed higher thigh yields than females. Similar results for breast and thigh yields have been reported by De Marchi et al. [26] in the *Padovana* breed (slaughtered at 150 or 180 days of age) and Tasoniero et al. [29] in the *Padovana* and *Polverara* breeds (slaughtered at 183 days of age). Moreover, Baéza et al. [28] found the same differences between sexes in the *Geline de Touraine*, the *Label rouge* genotype, and in an experimental crossbreed. Indeed, Mignon-Grasteau and Beaumont [30] reported that these differences were related to the precociousness of females over males. Further confirmation came from analysis of the cross-sectional area of muscle fibers, a measure that is directly related to bird age, and which was found to be higher in females than in males when compared at completed body development [28].

Meat color is considered to play a fundamental role in consumer choice, being an important selection criterion for poultry meat and meat products [31]. Consumers generally prefer their poultry meat to have a white or pale tan to pink color [31–33]. From a commercial point of view, the meat color of BS and BP breeds could be exploited as a distinctive trait, thus, creating a specific market niche. This objective has already been achieved in some Asian countries. For example, Pongduang et al. [34] demonstrated that red poultry meat with yellow-colored skin had become a feature highly appreciated by consumers in such areas.

The breast meat of both BP and BS females displayed greater lightness than that of male birds, and the value of this parameter also decreased over time in the BS males. On the contrary, Baéza et al. [28] reported no significant differences between sexes in the *Geline de Touraine*, the *Label rouge* genotype, or in an experimental crossbreed.

In the current study, the value of breast meat redness was always greater in male birds as compared with females. In males, breast redness increased as the birds became older, and this could be related to a higher concentration of haeminic pigments that increase with the age of the animals [28,35,36].

In this study, female birds generally showed higher levels of breast yellowness as compared with males. Moreover, differences in breast yellowness were observed in relation to breed. In particular, the breast meat of BP females presented the highest values of yellowness than any other birds at 6 months of age. This could be related both to gender and genetic traits that allow for a higher storage of pigments (such as carotenoids) in fat, which are mainly responsible for the yellow color of poultry meat [28,31,37].

Rizzi et al. [38] reported higher lightness values for the breast meat from *Ermellinata di Rovigo*, *Robusta Lionata,* or *Robusta Maculata* than those obtained in BP or BS breeds at 5–6 months of age. On the contrary, BP and BS breeds showed higher levels of yellowness and redness as compared with the abovementioned breeds [38]. By contrast, higher values in breast lightness, redness, and yellowness were reported for the BP and BS breeds than the *Polverara* and *Padovana* genotypes [29].

Meat color is also related to muscle pH, with a negative trend between the light reflecting properties of meat and pH [39]. Muscle pH also affects the meat's water holding capacity as well as meat composition [40]. Indeed, low pH values (near to 5.2) are responsible for lower water-holding capacities, being close to the meat isoelectric point that inhibits the capacity of protein to attract water [41]. Moreover, higher pH values have been pointed out as being useful for maintaining favorable meat color and moisture adsorption ability [41].

In the present study, the females of both breeds showed the highest pH values in thigh meat from birds aged 7 months, with the BP genotype associated with higher thigh pH values than the BS genotype.

In this research, the thigh pH values obtained (measured 24 h post mortem) were similar to those found by Devatkal et al. [42] in a slow growing genotype and commercial broilers. Tasoniero et al. [29] reported similar results in terms of breast pH in *Polverara* and *Padovana* breeds (48 h post mortem), while lower values were reported for thigh meat.

With regard to breast drip losses, our results revealed significantly higher values in males at 7 and 8 months of age despite there being no significant differences in terms of breast pH between breeds or genders.

Meat moisture plays a fundamental role in meat quality, being responsible for its perceived juiciness [43]. The moisture content of breast and thigh meat from the BP and BS breeds were similar to those reported by Bogosavljevic-Boskovic et al. [44] for broiler chickens raised in extensive indoor and free-range rearing systems. Interestingly, the moisture content of thigh meat was higher at 5 months of age than at the other slaughter ages considered. This may be related to the increased physical activity and fighting of birds close to sexual maturity that could increase the tonicity of the meat, or could be due to less time spent feeding and consuming water, resulting in the drop in meat moisture content [45]. Dalle Zotte et al. [14] reported similar values of breast meat moisture content in *Polverara* and *Padovana* breeds slaughtered at 6 months of age and BP and BS breeds slaughtered at a similar age.

The CP content of breast meat was only significantly higher in males as compared with in females at 5 months of age. Contrastingly, De Marchi et al. [26] reported similar breast CP content values between male and female *Polverara* and *Padovana* breeds, despite evidence of continuous tissue protein deposition that persisted beyond 5 months of age.

The CF and CP contents of meat have been reported to be affected by chicken strain, developmental growth rate, and bird gender [44]. In the present work, the CF content of thigh meat was higher than that of the breast, and was always higher in females than in males. This corroborates other work showing females to be richer in CF content than males, probably due to their faster tissue growth and rate of fat deposition [28,30,44,46]. Moreover, the important role of oestrogen in promoting lipid synthesis and deposition during the sexual maturity of birds must be considered as underlying the higher CF content of female meat [28].

Hydroxyproline is a secondary amino acid and is found almost exclusively in the connective tissue protein collagen. Consequently, the collagen content of meat can be measured indirectly by measuring hydroxyproline [47]. The HP concentration was significantly higher in BP females and BS males at 7 and 8 months of age. Chuaynukool et al. [48] explained that breed and/or age at slaughter might result in different collagen content levels in chicken meat. The same authors showed that meat from the *Thai* chicken breed at the market age of 16 weeks contained higher total collagen than those of 38-day commercial broilers [48]. Similar findings showing a high collagen content in *Thai* chickens [49] and Korean native chickens [50] as compared with other imported breeds has been reported in the literature. Similarly, Rajkumar et al. [51] showed a higher HP content in *Aseel* chickens as compared with broiler chicken meat at 6 months of age.

Dal Bosco et al. [52] demonstrated differences in meat FA composition in relation to different genotypes. In our study, breed and gender had a significant impact on breast SFA, MUFA, and PUFA contents. The predominant SFAs, MUFAs, and PUFAs found in BS and BP breast meat were the same as those found in five native African chicken genotypes [53].

Dal Bosco et al. [52] reported the BP and BS breeds to have a lower SFA content in breast meat as compared with other slow and medium growing chickens (*Leghorn*, *Ancona*, *Cornish* × *Leghorn*, and *Kabir*). Among the SFAs assessed, breast meat from BP and BS was higher in both palmitic and arachidonic acids as compared with the *Arbor Acres* broiler, the *Chinese* crossbred chicken, and *Hyline* hens [53,54]. Popova et al. [55], however, did not report an overall increase in breast SFA content related to line and age in two lines of slow growing chickens (*La Belle* and *Plymouth Rock*, slaughtered at 9 and 18 weeks of age), although the authors did report an increase in palmitic acid content. By contrast, the BP and BS breeds in the present study did not show any consistent increase in palmitic acid content with age, but a significant increase in total SFA content was recorded in the male birds of both breeds, and the values in males were higher than those in females. This could be related to the shown increase in stearic acid over time, and be due to the fact that the breast stearic acid content was higher in males than in females. These differences between sexes were also reported by Baéza et al. [28].

BP and BS breast meat presented lower levels of PUFA and higher values of MUFA as compared with those of all the above mentioned genotypes. The breast oleic acid content of the BP and BS breeds was similar to that of *Arbor Acres* broilers but lower than that shown in the *Chinese* crossbreed chicken, whereas the linoleic acid content was lower than those reported for all the genotypes studied by Chen et al. [54].

Interestingly, Cerolini et al. [45] reported similar results in male *Milanino* chickens, with an increase in SFA over time, an increase in PUFA content, and a decrease in MUFA content. In the male birds of both breeds in our study, the PUFA content decreased, whereas the MUFA content increased as slaughter age increased.

At all slaughter ages considered, females showed a higher breast MUFA content and a lower breast PUFA content than males. Similar results were observed in the *Géline de Touraine*, *Label rouge,* and an experimental crossbreed studied by Baéza et al. [28]. Klaising [56] reported that birds could synthetize MUFAs, such as oleic and palmitoleic acids. Since female birds are more precocious than males in terms of growth, MUFA synthesis and deposition in the adipose tissue may occur earlier on than in male birds.

The differences in fatty acid content in breast meat in relation to breed, gender, and slaughter age could be useful to identify the best slaughter period in order to obtain the most favorable proportion of fatty acids for human health. This balance is recognized as 1:1.5:1 (SFA/MUFA/PUFA), and is fundamental in order to generate the best LDL/HDL ratio [57]. Based on the results obtained in the present work, the most favorable slaughter age in terms of FA composition in breast meat could be identified at 5 months of age, when the PUFA content was at its highest as compared with the other months studied.

Regarding thigh FA composition, the SFA and MUFA contents were higher than those of breast meat, whereas the PUFA content was lower, due to the lower proportion of linoleic and arachidonic acids.

In the present work, the males were higher in thigh SFA content than females at every slaughter age studied, despite palmitic acid always being higher in females. By contrast, the males presented a higher proportion of stearic acid with respect to females at every age. The BP breed contained more palmitic acid than the BS breed, particularly at 5 months of age, whereas the BS breed contained more stearic acid than the BP breed at this age.

In contrast with the results of the present work, Popova et al. [55] reported that the SFA content in thigh meat was significantly increased in older birds. Chen et al. [54] reported on the FA composition of thigh meat in a Chinese crossbred chicken and *Arbor Acres* broiler, and interestingly, they found lower levels of thigh SFA in these genotypes as compared with those found in the BP and BS breeds. This could be related to the slaughter age of birds that was 45 and 40 days of age for the Chinese crossbred chicken and the *Arbor Acres* broiler, respectively [54]. Therefore, these birds were younger than the BP and BS breeds at the moment of slaughter, and a higher deposition of fat in older birds as compared with younger birds is a well-known fact [58]. Such differences in fat content and FA composition could be explained by the genotype effect that, according to Tang et al. [59], could have a more relevant influence on fat deposition than age of slaughter. Despite differences in total SFA content, the proportion of the palmitic acid content was always greater than that of stearic acid, as also found by Tang et al. [59] and Chen et al. [54].

In general, female birds showed higher thigh MUFA content than males. The BS genotype was higher in MUFA content than the BP genotype at 7 months of age due to increased oleic acid content. The MUFA content in BP and BS genotypes was similar to that of the Chinese crossbreed chicken, but lower than that of the *Arbor Acres* broiler as reported by Chen et al. [54].

Independently of breed, the PUFA content was always higher in males as compared with females. This result lies in accordance with those reported by Cerolini et al. [45] for the *Milanino* chicken breed slaughtered at 7–8 months of age. Thigh PUFA content in female birds was highest at 5 months of age. This could be explained by a lower amount of total CF content at 5 months of age in females. The total CF content increased over time in contrast with the PUFA content that became more diluted. As compared with all the genotypes mentioned by Chen et al. [54], the BP and BS breeds of the present study showed much lower PUFA contents in thigh meat.

## 5. Conclusions

The results obtained in this study suggest that slaughtering at 7 or 8 months of age could present the best carcass parameters in BP birds of both genders and in male BS birds. In addition, 5 and 6 months of age would constitute the best slaughter ages for BS females in terms of carcass yield. Although there were no significant differences in terms of slaughtering weight, the BP genotype appears to be the more productive breed in terms of carcass yield as compared with the BS breed at 5, 7, and 8 months of age. A higher moisture content in thigh meat at 5 months was observed in all birds, and could have a positive influence on the parameter meat juiciness. As compared with the other studies, a lower SFA content and a greater MUFA content were observed in breast meat. Moreover, the BS genotype presented a lower SFA content in thigh meat than the BP genotype. From the health point of view, the breast meat of both BP and BS females was superior to that of their thigh meat due to its lower content of SFA [57].

These results could guide breeders to select the most favorable moment at which to slaughter BS and BP males and females, even though irregular trends were recorded between different slaughter ages in terms of their nutritional profiles and meat properties.

Further research should be conducted to define a stable niche market for BP and BS products and to preserve the genetic variability of these breeds over time. Sensorial analysis could be performed to identify a specific consumer profile and potentially identify any distinctive characteristics of BP and BS meat. Moreover, additional studies should be conducted on meat tenderness and juiciness of these two chicken breeds, since these parameters represent fundamental meat quality indicators for the consumers [60,61].

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani12030406/s1, Table S1: Slaughter yields of *Bionda Piemontese* and *Bianca di Saluzzo* breeds at different slaughter ages, Table S2: Meat quality parameters of breast and thigh muscles at different slaughter ages, Table S3: Chemical composition (g/100 g FM) and hydroxyproline content (mg/g dry weight) of breast meat at different slaughter ages, Table S4: Chemical composition (g/100 g FM) of thigh meat at different slaughter ages, Table S5: Saturated fatty acid profile of breast meat at different slaughter ages (g/100 g of total fatty acids), Table S6: Monounsaturated fatty acid profile of breast meat at different slaughter ages (g/100 g of total fatty acids), Table S7: Polyunsaturated fatty acid profile of breast meat at different slaughter ages (g/100 g of total fatty acids), Table S8: Saturated fatty acid profile of thigh meat at different slaughter ages (g/100 g of total fatty acids), Table S9: Monounsaturated fatty acid profile of thigh meat at different slaughter ages (g/100 g of total fatty acids), Table S10: Polyunsaturated fatty acid profile of thigh meat at different slaughter ages (g/100 g of total fatty acids).

**Author Contributions:** Conceptualization, A.S.; methodology, A.S., A.C., S.D., A.B., S.M., P.S. and F.S.; software, S.D.; validation, A.S., L.G. and M.R.; formal analysis, S.D., C.B., A.C., S.M. and M.Z.; investigation, A.S., M.M. and S.C.; Sample analysis, A.S., C.B., M.R., S.S., D.S., A.B., F.G. and S.D.; data curation, S.D.; writing—original draft preparation, V.B., S.D. and M.G.; writing—review and editing, V.B., M.G., S.D. and A.S.; visualization, A.S., C.M., M.R., C.F., I.B., L.G. and F.G.; supervision, A.S.; project administration, D.S.; funding acquisition, D.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by CoVaRAP—Conservazione e valorizzazione delle razze avicole del Piemonte (Italy)-Fondi Regione Piemonte PSR 2014-2020—16.2.1—Az. 2—Progetti pilota—Piattaforma tecnologica bioeconomia (Research agreement n. CUP-J62C21002570002).

**Institutional Review Board Statement:** The study was approved by the Bioethical Committee of the University of Turin (Italy) (reference no. 814715, 23 July 2019). All animals were handled according to the principles stated by the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** The authors are grateful to Paolo Montersino, Dario Sola, and Mario Colombano for the bird care and technical support.

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

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# Carcass Yields and Meat Composition of Male and Female Italian Slow-Growing Chicken Breeds: *Bianca di Saluzzo* and *Bionda Piemontese*

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*Animals* **2022**, *12*, 406. https://doi.org/10.3390/ani12040406 www.mdpi.com/journal/animals

**Supplementary materials**

**Table S1.** Slaughter yields of *Bionda Piemontese* and *Bianca di Saluzzo* breeds at different slaughter ages (n = 8).

| Items | Age at slaughter | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | | Breed | Gender |
| Carcass yield (% SW) | 5 months | 60.03 | 60.47 | 57.96 | 59.03$^{a}$ | 0.431 | 0.028 | 0.326 |
| | 6 months | 60.58 | 59.33 | 59.38 | 57.58$^{ab}$ | 0.490 | 0.131 | 0.119 |
| | 7 months | 61.58 | 57.54 | 59.20 | 54.21$^{bc}$ | 0.761 | 0.029 | 0.001 |
| | 8 months | 58.92 | 56.78 | 57.22 | 52.72$^{c}$ | 0.678 | 0.017 | 0.007 |
| ***p*-value** | | 0.080 | 0.083 | 0.122 | 0.020 | | | |
| Breast yield (%CC) | 5 months | 16.16$^{b}$ | 19.09$^{a}$ | 17.76$^{a}$ | 19.02$^{b}$ | 0.285 | 0.064 | <0.001 |
| | 6 months | 15.45$^{b}$ | 17.39$^{c}$ | 15.65$^{b}$ | 16.87$^{c}$ | 0.228 | 0.658 | <0.001 |
| | 7 months | 17.50$^{a}$ | 18.92$^{bc}$ | 17.72$^{a}$ | 19.63$^{ab}$ | 0.290 | 0.371 | 0.003 |
| | 8 months | 18.09$^{a}$ | 20.99$^{a}$ | 17.91$^{a}$ | 20.67$^{a}$ | 0.324 | 0.556 | <0.001 |
| ***p*-value** | | <0.001 | 0.002 | 0.002 | <0.001 | | | |
| Thigh yield (%CC) | 5 months | 38.89 | 33.23 | 38.56 | 32.67 | 0.626 | 0.544 | <0.001 |
| | 6 months | 39.03 | 32.46 | 38.41 | 31.59 | 0.654 | 0.118 | <0.001 |
| | 7 months | 38.51 | 32.17 | 38.93 | 32.25 | 0.621 | 0.573 | <0.001 |
| | 8 months | 39.95 | 32.41 | 39.96 | 32.39 | 0.714 | 0.989 | <0.001 |
| ***p*-value** | | 0.385 | 0.513 | 0.230 | 0.480 | | | |

Abbreviations: SW: slaughter weight; CC: chilled carcass weight; SEM: standard error of the mean; values with different superscript letters (a, b, c) within the same column per fixed effect (age of slaughter) differ significantly ($p < 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

**Table S2.** Meat quality parameters of breast and thigh muscles at different slaughter ages (n = 8).

| Items | Age at slaughter | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | | Breed | Gender |
| pH breast | 5 months | 6.21 | 6.10$^{b}$ | 6.34 | 6.15$^{c}$ | 0.035 | 0.189 | 0.034 |
| | 6 months | 6.18 | 6.25$^{b}$ | 6.21 | 6.24$^{ab}$ | 0.019 | 0.823 | 0.187 |
| | 7 months | 6.22 | 6.45$^{a}$ | 6.22 | 6.21$^{c}$ | 0.031 | 0.034 | 0.052 |
| | 8 months | 6.21 | 6.24$^{b}$ | 6.31 | 6.35$^{a}$ | 0.037 | 0.185 | 0.650 |
| ***p*-value** | | 0.974 | 0.002 | 0.512 | 0.015 | | | |
| pH thigh | 5 months | 6.21 | 6.10$^{b}$ | 6.34 | 6.15$^{c}$ | 0.035 | 0.189 | 0.034 |
| | 6 months | 6.18 | 6.25$^{b}$ | 6.21 | 6.24$^{ab}$ | 0.019 | 0.823 | 0.187 |
| | 7 months | 6.22 | 6.45$^{a}$ | 6.22 | 6.21$^{c}$ | 0.031 | 0.034 | 0.052 |
| | 8 months | 6.21 | 6.24$^{b}$ | 6.31 | 6.35$^{a}$ | 0.037 | 0.185 | 0.650 |
| ***p*-value** | | 0.974 | 0.002 | 0.512 | 0.015 | | | |
| | | | Breast meat | | | | | |
| Lightness (L*) | 5 months | 51.69 | 53.98 | 52.54$^{a}$ | 53.66 | 0.514 | 0.800 | 0.108 |
| | 6 months | 50.10 | 53.15 | 50.98$^{a}$ | 54.45 | 0.503 | 0.200 | 0.001 |
| | 7 months | 52.96 | 55.05 | 53.47$^{a}$ | 54.03 | 0.403 | 0.750 | 0.106 |
| | 8 months | 50.12 | 53.42 | 47.55$^{b}$ | 54.36 | 0.644 | 0.366 | <0.001 |
| ***p*-value** | | 0.102 | 0.447 | <0.001 | 0.931 | | | |
| Redness (a*) | 5 months | -0.23$^{c}$ | -1.64b | -0.30$^{b}$ | -1.95$^{b}$ | 0.221 | 0.608 | <0.001 |
| | 6 months | 1.24$^{b}$ | 0.32$^{a}$ | 0.26$^{b}$ | -0,13$^{a}$ | 0.278 | 0.205 | 0.248 |
| | 7 months | 2.01$^{b}$ | -0.17$^{a}$ | 1.17$^{b}$ | -0.68$^{a}$ | 0.264 | 0.089 | <0.001 |
| | 8 months | 4.55$^{a}$ | 1.28$^{a}$ | 4.48$^{a}$ | 0.32$^{a}$ | 0.416 | 0.314 | <0.001 |
| ***p*-value** | | <0.001 | 0.003 | <0.001 | 0.001 | | | |
| Yellowness (b*) | 5 months | 7.95 | 9.23 | 7.68ab | 7.68 | 0.274 | 0.092 | 0.231 |
| | 6 months | 7.99 | 11.01 | 6.24b | 9.74 | 0.418 | 0.012 | <0.001 |
| | 7 months | 8.35 | 10.06 | 9.54$^{a}$ | 9.67 | 0.394 | 0.619 | 0.260 |
| | 8 months | 10.18 | 11.29 | 8.96$^{a}$ | 10.33 | 0.426 | 0.206 | 0.151 |
| ***p*-value** | | 0.143 | 0.150 | 0.006 | 0.061 | | | |
| Drip losses (%) | 5 months | 13.82$^{a}$ | 11.38$^{a}$ | 12.15$^{a}$ | 11.77$^{a}$ | 0.542 | 0.557 | 0.205 |
| | 6 months | 7.86$^{b}$ | 7.52$^{b}$ | 7.05$^{b}$ | 7.01$^{b}$ | 0.218 | 0.146 | 0.666 |
| | 7 months | 6.48$^{b}$ | 4.08$^{c}$ | 6.78$^{b}$ | 4.32$^{c}$ | 0.306 | 0.543 | <0.001 |
| | 8 months | 4.31$^{c}$ | 3.26$^{c}$ | 3.85$^{c}$ | 3.19$^{c}$ | 0.132 | 0.224 | 0.001 |
| ***p*-value** | | <0.001 | <0.001 | <0.001 | <0.001 | | | |

Abbreviations: SEM: standard error of the mean; values with different superscript letters (a, b, c) within the same column per fixed effect (age of slaughter) differ significantly ($p < 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

**Table S3.** Chemical composition (g/100 g FM) and hydroxyproline content (mg/g dry weight) of breast meat at different slaughter ages (n = 8).

| Items | Age at slaughter | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | | Breed | Gender |
| Moisture | 5 months | 75.13$^{a}$ | 73.22$^{ab}$ | 74.98$^{a}$ | 73.38 | 0.227 | 0.984 | <0.001 |
| | 6 months | 74.63$^{ab}$ | 74.08$^{a}$ | 74.37$^{ab}$ | 73.82 | 0.119 | 0.254 | 0.020 |
| | 7 months | 73.90$^{bc}$ | 72.53$^{c}$ | 73.55$^{b}$ | 73.06 | 0.162 | 0.740 | 0.002 |
| | 8 months | 73.75$^{c}$ | 73.47$^{ab}$ | 73.79$^{b}$ | 73.42 | 0.119 | 0.998 | 0.192 |
| ***p*-value** | | 0.03 | 0.03 | 0.011 | 0.294 | | | |
| Crude protein | 5 months | 26.13 | 24.30 | 26.66$^{a}$ | 25.20 | 0.325 | 0.235 | 0.010 |
| | 6 months | 24.20 | 24.62 | 24.36$^{c}$ | 24.52 | 0.115 | 0.901 | 0.223 |
| | 7 months | 24.73 | 25.18 | 25.96$^{ab}$ | 24.93 | 0.220 | 0.264 | 0.514 |
| | 8 months | 24.95 | 24.82 | 25.31$^{ab}$ | 24.72 | 0.136 | 0.642 | 0.197 |
| ***p*-value** | | 0.071 | 0.489 | 0.006 | 0.330 | | | |
| Crude fat | 5 months | 0.23 | 0.75$^{b}$ | 0.11$^{b}$ | 0.55$^{b}$ | 0.069 | 0.147 | <0.001 |
| | 6 months | 0.31 | 0.61$^{b}$ | 0.29$^{a}$ | 0.88$^{ab}$ | 0.087 | 0.429 | 0.009 |
| | 7 months | 0.33 | 1.72$^{a}$ | 0.30$^{a}$ | 1.22$^{a}$ | 0.138 | 0.162 | <0.001 |
| | 8 months | 0.37 | 0.50$^{b}$ | 0.21$^{ab}$ | 0.35$^{b}$ | 0.038 | 0.033 | 0.056 |
| ***p*-value** | | 0.329 | <0.001 | 0.046 | 0.014 | | | |
| Ash | 5 months | 1.14 | 1.22 | 1.13$^{b}$ | 1.22 | 0.013 | 0.819 | 0.004 |
| | 6 months | 1.17 | 1.20 | 1.15$^{b}$ | 1.15 | 0.008 | 0.048 | 0.301 |
| | 7 months | 1.19 | 1.18 | 1.21$^{a}$ | 1.16 | 0.010 | 1.000 | 0.260 |
| | 8 months | 1.21 | 1.20 | 1.18$^{ab}$ | 1.21 | 0.009 | 0.767 | 0.576 |
| ***p*-value** | | 0.250 | 0.769 | 0.036 | 0.065 | | | |
| Hydroxy proline | 5 months | 1.49 | 2.33$^{ab}$ | 1.12$^{c}$ | 2.48 | 0.179 | 0.722 | 0.001 |
| | 6 months | 1.95 | 1.84$^{b}$ | 1.98$^{bc}$ | 2.13 | 0.102 | 0.454 | 0.918 |
| | 7 months | 2.75 | 3.02$^{a}$ | 3.06$^{a}$ | 3.04 | 0.223 | 0.500 | 0.956 |
| | 8 months | 2.99 | 3.00$^{a}$ | 2.89$^{ab}$ | 3.16 | 0.169 | 0.903 | 0.668 |
| ***p*-value** | | 0.075 | 0.013 | <0.001 | 0.107 | | | |

Abbreviations: FM: fresh matter; SEM: standard error of the mean; values with different superscript letters (a, b, c) within the same column per fixed effect (age of slaughter) differ significantly ($p < 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

**Table S4.** Chemical composition (g/100 g FM) of thigh meat at different slaughter ages (n = 8).

| Items | Age at slaughter | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | | Breed | Gender |
| Moisture | 5 months | 76.19[a] | 82.21[a] | 76.42[a] | 81.47[a] | 0.740 | 0.827 | <0.001 |
| | 6 months | 74.96[b] | 73.39[b] | 75.04[b] | 73.12[b] | 0.211 | 0.752 | <0.001 |
| | 7 months | 75.26[b] | 71.54[b] | 75.08[b] | 71.84[b] | 0.346 | 0.854 | <0.001 |
| | 8 months | 74.76[b] | 72.57[b] | 75.12[b] | 72.14[b] | 0.329 | 0.940 | <0.001 |
| ***p*-value** | | 0.004 | <0.001 | 0.003 | <0.001 | | | |
| Crude protein | 5 months | 26.85 | 26.88 | 27.13 | 27.89 | 0.303 | 0.308 | 0.526 |
| | 6 months | 27.11 | 26.35 | 27.38 | 26.19 | 0.186 | 0.873 | 0.008 |
| | 7 months | 27.58 | 28.15 | 27.81 | 29.33 | 0.298 | 0.416 | 0.232 |
| | 8 months | 27.36 | 27.98 | 27.68 | 28.72 | 0.417 | 0.540 | 0.340 |
| ***p*-value** | | 0.682 | 0.359 | 0.747 | 0.018 | | | |
| Crude fat extract | 5 months | 1.00[b] | 2.30[c] | 0.96[c] | 2.28c | 0.136 | 0.859 | <0.001 |
| | 6 months | 2.15[a] | 4.33[b] | 2.55[a] | 4.91[b] | 0.274 | 0.194 | <0.001 |
| | 7 months | 1.65[a] | 6.92[a] | 1.79[b] | 6.14[a] | 0.460 | 0.325 | <0.001 |
| | 8 months | 2.03[a] | 4.28[b] | 1.84[b] | 4.01[b] | 0.220 | 0.246 | <0.001 |
| ***p*-value** | | <0.001 | <0.001 | <0.001 | <0.001 | | | |
| Ash | 5 months | 1.08 | 0.75[b] | 1.08 | 0.77[c] | 0.038 | 0.838 | <0.001 |
| | 6 months | 1.13 | 1.12[a] | 1.12 | 1.08[ab] | 0.009 | 0.227 | 0.121 |
| | 7 months | 1.10 | 1.02[a] | 1.07 | 1.01[b] | 0.008 | 0.035 | <0.001 |
| | 8 months | 1.13 | 1.13[a] | 1.11 | 1.14[a] | 0.011 | 0.937 | 0.400 |
| ***p*-value** | | 0.060 | <0.001 | 0.066 | <0.001 | | | |

Abbreviations: FM: fresh matter; SEM: standard error of the mean; values with different superscript letters (a, b, c) within the same column per fixed effect (age of slaughter) differ significantly ($p< 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

**Table S5.** Saturated fatty acid profile of breast meat at different slaughter ages (g/100g of total fatty acids) (n = 8).

| Items | Age at slaughter | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | | Breed | Gender |
| C14 | 5 months | 0.36 | 0.60$^{b}$ | 0.13 | 0.41$^{b}$ | 0.049 | 0.014 | 0.003 |
| | 6 months | 0.39 | 0.51$^{b}$ | 0.18 | 0.48$^{b}$ | 0.034 | 0.032 | 0.001 |
| | 7 months | 0.41 | 0.86$^{a}$ | 0.32 | 0.66$^{a}$ | 0.049 | 0.042 | <0.001 |
| | 8 months | 0.51 | 0.59$^{b}$ | 1.02 | 0.50$^{b}$ | 0.191 | 0.549 | 0.571 |
| ***p*-value** | | 0.531 | 0.001 | 0.362 | 0.023 | | | |
| C16 | 5 months | 24.35$^{b}$ | 28.43$^{bc}$ | 24.72 | 26.36 | 0.580 | 0.427 | 0.427 |
| | 6 months | 27.74$^{a}$ | 26.74$^{c}$ | 27.09 | 27.60 | 0.421 | 0.905 | 0.781 |
| | 7 months | 30.05$^{a}$ | 33.27$^{a}$ | 27.07 | 29.10 | 0.656 | 0.003 | 0.023 |
| | 8 months | 30.41$^{a}$ | 29.20$^{b}$ | 28.88 | 27.97 | 0.417 | 0.101 | 0.204 |
| ***p*-value** | | 0.001 | <0.001 | 0.171 | 0.103 | | | |
| C17 | 5 months | 0.24 | 0.32$^{ab}$ | 0.16 | 0.26 | 0.024 | 0.133 | 0.072 |
| | 6 months | 0.25 | 0.20$^{c}$ | 0.16 | 0.30 | 0.022 | 0.942 | 0.269 |
| | 7 months | 0.48 | 0.41$^{a}$ | 0.21 | 0.40 | 0.039 | 0.062 | 0.375 |
| | 8 months | 0.37 | 0.31$^{b}$ | 0.43 | 0.29 | 0.058 | 0.878 | 0.394 |
| ***p*-value** | | 0.123 | 0.003 | 0.352 | 0.106 | | | |
| C18 | 5 months | 12.79$^{b}$ | 12.77$^{a}$ | 14.06$^{b}$ | 12.73$^{a}$ | 0.249 | 0.207 | 0.168 |
| | 6 months | 13.74$^{b}$ | 11.26$^{b}$ | 13.60$^{b}$ | 12.58$^{a}$ | 0.250 | 0.119 | <0.001 |
| | 7 months | 15.43$^{a}$ | 11.51$^{b}$ | 14.52$^{b}$ | 10.84$^{b}$ | 0.393 | 0.046 | <0.001 |
| | 8 months | 14.96$^{a}$ | 10.91$^{b}$ | 16.72$^{a}$ | 11.98$^{a}$ | 0.445 | <0.001 | <0.001 |
| ***p*-value** | | <0.001 | 0.011 | <0.001 | 0.006 | | | |
| C24 | 5 months | 1.56$^{a}$ | 1.06 | 2.04 | 1.58$^{a}$ | 0.097 | 0.004 | 0.005 |
| | 6 months | 1.20$^{ab}$ | 1.27 | 1.52 | 0.90$^{b}$ | 0.127 | 0.924 | 0.283 |
| | 7 months | 0.87$^{bc}$ | 0.66 | 1.12 | 0.35$^{c}$ | 0.103 | 0.894 | 0.015 |
| | 8 months | 0.67$^{c}$ | 1.15 | 1.15 | 0.78$^{b}$ | 0.087 | 0.725 | 0.736 |
| ***p*-value** | | 0.002 | 0.232 | 0.065 | <0.001 | | | |
| ΣSFA | 5 months | 39.30$^{c}$ | 43.18$^{b}$ | 41.11$^{b}$ | 41.33 | 0.646 | 0.987 | 0.114 |
| | 6 months | 43.33$^{b}$ | 39.98$^{c}$ | 42.55$^{b}$ | 41.87 | 0.487 | 0.547 | 0.035 |
| | 7 months | 47.24$^{a}$ | 46.71$^{a}$ | 43.25$^{b}$ | 41.36 | 0.573 | 0.001 | 0.353 |
| | 8 months | 46.93$^{a}$ | 42.16$^{bc}$ | 48.21$^{a}$ | 41.53 | 0.677 | 0.720 | <0.001 |
| ***p*-value** | | <0.001 | <0.001 | 0.010 | 0.973 | | | |

Abbreviations: SFA: saturated fatty acids; SEM: standard error of the mean; values with different superscript letters (a, b, c) within the same column per fixed effect (age of slaughter) differ significantly ($p < 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

**Table S6.** Monounsaturated fatty acid profile of breast meat at different slaughter ages (g/100g of total detected fatty acids) (n = 8).

| Items | Age at slaughter | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | | Breed | Gender |
| C16 :1n7 | 5 months | 1.23 | 1.56$^{b}$ | 0.69 | 1.51$^{b}$ | 0.132 | 0.244 | 0.027 |
| | 6 months | 1.07 | 1.95$^{b}$ | 1.23 | 1.80$^{b}$ | 0.134 | 0.986 | 0.006 |
| | 7 months | 1.17 | 3.71$^{a}$ | 1.10 | 3.24$^{a}$ | 0.239 | 0.260 | <0.001 |
| | 8 months | 1.34 | 2.16$^{b}$ | 0.56 | 1.81$^{b}$ | 0.147 | 0.012 | <0.001 |
| ***p*-value** | | 0.846 | <0.001 | 0.056 | <0.001 | | | |
| C18 :1c9 | 5 months | 25.46$^{b}$ | 31.20$^{c}$ | 23.34$^{b}$ | 29.87$^{c}$ | 0.804 | 0.157 | <0.001 |
| | 6 months | 28.40$^{ab}$ | 31.39$^{c}$ | 30.07$^{a}$ | 32.39$^{bc}$ | 0.706 | 0.340 | 0.063 |
| | 7 months | 29.43$^{a}$ | 40.48$^{a}$ | 30.82$^{a}$ | 40.78$^{a}$ | 1.097 | 0.474 | <0.001 |
| | 8 months | 30.79$^{a}$ | 36.95$^{b}$ | 28.49$^{a}$ | 34.20$^{b}$ | 0.796 | 0.036 | <0.001 |
| ***p*-value** | | 0.033 | <0.001 | 0.001 | <0.001 | | | |
| C20: 1 | 5 months | 0.20 | 0.18 | 0.06 | 0.28 | 0.030 | 0.756 | 0.071 |
| | 6 months | 0.38 | 0.15 | 0.09 | 0.34 | 0.037 | 0.392 | 0.913 |
| | 7 months | 0.23 | 0.26 | 0.26 | 0.29 | 0.034 | 0.681 | 0.681 |
| | 8 months | 0.27 | 0.21 | 0.20 | 0.19 | 0.032 | 0.537 | 0.574 |
| ***p*-value** | | 0.224 | 0.605 | 0.229 | 0.403 | | | |
| C24:1 | 5 months | 0.58 | 0.64$^{a}$ | 0.98 | 0.53$^{a}$ | 0.072 | 0.289 | 0.159 |
| | 6 months | 0.33 | 0.70$^{a}$ | 0.62 | 0.59$^{a}$ | 0.065 | 0.498 | 0.179 |
| | 7 months | 0.56 | 0.15$^{b}$ | 0.42 | 0.21$^{b}$ | 0.088 | 0.808 | 0.085 |
| | 8 months | 0.21 | 0.51$^{a}$ | 0.72 | 0.68$^{a}$ | 0.065 | 0.006 | 0.260 |
| ***p*-value** | | 0.384 | 0.013 | 0.090 | 0.006 | | | |
| ΣMUFA | 5 months | 27.46 | 33.58$^{c}$ | 25.08$^{b}$ | 32.20$^{c}$ | 0.871 | 0.155 | <0.001 |
| | 6 months | 30.18 | 34.19$^{c}$ | 32.01$^{a}$ | 35.12$^{bc}$ | 0.812 | 0.381 | 0.029 |
| | 7 months | 31.38 | 44.60$^{a}$ | 32.60$^{a}$ | 44.52$^{a}$ | 0.774 | 0.674 | <0.001 |
| | 8 months | 32.62 | 39.83$^{b}$ | 29.97$^{a}$ | 36.89$^{b}$ | 0.902 | 0.033 | <0.001 |
| ***p*-value** | | 0.087 | <0.001 | 0.003 | <0.001 | | | |

Abbreviations: MUFA: monounsaturated fatty acids; SEM: standard error of the mean; values with different superscript letters (a, b, c) within the same column per fixed effect (age of slaughter) differ significantly ($p < 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

**Table S7.** Polyunsaturated fatty acid profile of breast meat at different slaughter ages (g/100g of total detected fatty acids) (n = 8).

| Items | Age at slaughter | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | | Breed | Gender |
| C18 :2n6 | 5 months | 15.18 | 13.07$^{a}$ | 15.00 | 13.47 | 0.476 | 0.908 | 0.063 |
| | 6 months | 12.97 | 13.95$^{a}$ | 11.47 | 11.75 | 0.369 | 0.011 | 0.359 |
| | 7 months | 11.71 | 4.71$^{b}$ | 12.69 | 9.49 | 0.762 | 0.015 | <0.001 |
| | 8 months | 13.67 | 11.90$^{a}$ | 13.37 | 11.22 | 0.567 | 0.666 | 0.094 |
| ***p*-value** | | 0.256 | <0.001 | 0.056 | 0.060 | | | |
| C20 :2n6 | 5 months | 0.32 | 0.16$^{ab}$ | 0.19 | 0.24 | 0.035 | 0.700 | 0.438 |
| | 6 months | 0.25 | 0.11$^{b}$ | 0.37 | 0.13 | 0.369 | 0.211 | 0.002 |
| | 7 months | 0.22 | 0.03$^{b}$ | 0.21 | 0.12 | 0.030 | 0.458 | 0.012 |
| | 8 months | 0.23 | 0.28$^{a}$ | 0.39 | 0.18 | 0.037 | 0.735 | 0.276 |
| ***p*-value** | | 0.631 | 0.009 | 0.224 | 0.376 | | | |
| C20: 4n6 | 5 months | 10.19$^{a}$ | 6.86$^{a}$ | 12.67$^{a}$ | 8.06$^{a}$ | 0.578 | 0.047 | <0.001 |
| | 6 months | 7.72$^{a}$ | 7.35$^{a}$ | 8.32$^{b}$ | 6.24$^{ab}$ | 0.501 | 0.803 | 0.243 |
| | 7 months | 4.69$^{b}$ | 0.65$^{b}$ | 5.74$^{b}$ | 1.84$^{c}$ | 0.610 | 0.282 | 0.001 |
| | 8 months | 2.81$^{b}$ | 1.47$^{b}$ | 1.48$^{c}$ | 4.63$^{b}$ | 0.483 | 0.313 | 0.320 |
| ***p*-value** | | <0.001 | <0.001 | <0.001 | <0.001 | | | |
| Σn6 PUFA | 5 months | 25.69$^{a}$ | 20.09$^{a}$ | 27.85$^{a}$ | 21.77$^{a}$ | 0.909 | 0.217 | 0.004 |
| | 6 months | 20.94$^{b}$ | 21.43$^{a}$ | 20.15$^{b}$ | 18.25$^{ab}$ | 0.714 | 0.177 | 0.625 |
| | 7 months | 16.62$^{b}$ | 5.41$^{c}$ | 18.64$^{b}$ | 11.48$^{c}$ | 1.240 | 0.028 | <0.001 |
| | 8 months | 16.72$^{b}$ | 13.65$^{b}$ | 15.24$^{b}$ | 16.03$^{bc}$ | 0.822 | 0.792 | 0.503 |
| ***p*-value** | | 0.001 | <0.001 | <0.001 | 0.001 | | | |
| C18 :3n3 | 5 months | 0.06 | 0.11$^{b}$ | 0.00$^{b}$ | 0.15 | 0.018 | 0.759 | 0.004 |
| | 6 months | 0.06 | 0.11$^{b}$ | 0.03$^{b}$ | 0.13 | 0.021 | 0.840 | 0.109 |
| | 7 months | 0.09 | 0.10$^{b}$ | 0.14$^{a}$ | 0.19 | 0.020 | 0.109 | 0.506 |
| | 8 months | 0.12 | 0.54$^{a}$ | 0.02$^{b}$ | 0.20 | 0.056 | 0.024 | 0.003 |
| ***p*-value** | | 0.597 | 0.001 | 0.002 | 0.742 | | | |
| C20 :5n3 | 5 months | 1.21$^{a}$ | 0.36 | 0.71 | 0.40 | 0.201 | 0.571 | 0.159 |
| | 6 months | 0.22$^{b}$ | 0.44 | 0.61 | 0.62 | 0.158 | 0.389 | 0.735 |
| | 7 months | 0.06$^{b}$ | 0.01 | 0.50 | 0.01 | 0.061 | 0.039 | 0.014 |
| | 8 months | 0.31$^{b}$ | 0.12 | 1.00 | 0.30 | 0.166 | 0.193 | 0.179 |
| ***p*-value** | | 0.047 | 0.542 | 0.853 | 0.406 | | | |
| C22 :5n3 | 5 months | 1.08$^{a}$ | 0.65$^{a}$ | 1.25 | 0.70$^{a}$ | 0.092 | 0.513 | 0.007 |
| | 6 months | 0.60$^{b}$ | 0.52$^{a}$ | 0.85 | 0.54$^{a}$ | 0.061 | 0.269 | 0.114 |
| | 7 months | 0.40$^{b}$ | 0.05$^{b}$ | 0.77 | 0.15$^{b}$ | 0.087 | 0.124 | 0.003 |
| | 8 months | 0.36$^{b}$ | 0.27$^{b}$ | 0.84 | 0.49$^{a}$ | 0.166 | 0.004 | 0.060 |
| ***p*-value** | | 0.004 | <0.001 | 0.353 | 0.005 | | | |
| C22 :6n3 | 5 months | 0.95$^{a}$ | 0.87$^{ab}$ | 1.14 | 0.66$^{ab}$ | 0.086 | 0.956 | 0.104 |
| | 6 months | 0.56$^{b}$ | 1.15$^{a}$ | 0.72 | 0.73$^{ab}$ | 0.076 | 0.347 | 0.036 |
| | 7 months | 0.38$^{b}$ | 0.08$^{c}$ | 0.61 | 0.18$^{b}$ | 0.071 | 0.199 | 0.007 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 8 months | $0.31^{b}$ | $0.49^{bc}$ | 0.80 | $1.13^{a}$ | 0.127 | 0.027 | 0.296 |
| ***p*-value** | | 0.008 | <0.001 | 0.279 | 0.041 | | | |
| Σn3 PUFA | 5 months | $3.31^{a}$ | $1.98^{a}$ | 3.11 | $1.92^{a}$ | 0.280 | 0.805 | 0.028 |
| | 6 months | $1.45^{b}$ | $2.22^{a}$ | 2.21 | $2.03^{a}$ | 0.209 | 0.511 | 0.493 |
| | 7 months | $0.93^{b}$ | $0.24^{b}$ | 2.03 | $0.54^{b}$ | 0.168 | 0.008 | <0.001 |
| | 8 months | $1.10^{b}$ | $1.42^{a}$ | 2.66 | $2.11^{a}$ | 0.283 | 0.050 | 0.837 |
| ***p*-value** | | 0.002 | <0.001 | 0.641 | 0.021 | | | |
| ΣPUFA | 5 months | $29.01^{a}$ | $22.08^{a}$ | $30.96^{a}$ | $23.69^{a}$ | 1.041 | 0.303 | 0.002 |
| | 6 months | $22.39^{b}$ | $23.63^{a}$ | $22.36^{b}$ | $20.15^{ab}$ | 0.829 | 0.305 | 0.774 |
| | 7 months | $17.56^{b}$ | $5.63^{c}$ | $20.67^{b}$ | $11.98^{c}$ | 1.370 | 0.019 | <0.001 |
| | 8 months | $17.82^{b}$ | $15.08^{b}$ | $17.90^{b}$ | $18.14^{b}$ | 0.877 | 0.386 | 0.487 |
| ***p*-value** | | <0.001 | <0.001 | 0.001 | <0.001 | | | |

Abbreviations: PUFA: polyunsaturated fatty acids; SEM: standard error of the mean; values with different superscript letters (a, b, c) within the same column per fixed effect (age of slaughter) differ significantly ($p < 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

**Table S8.** Saturated fatty acid profile of thigh meat at different slaughter ages (g/100g of total fatty acids) (n = 8).

| Items | Age at slaughter | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | | Breed | Gender |
| C14 | 5 months | 0.97[a] | 1.26 | 0.86[a] | 1.01 | 0.049 | 0.038 | 0.015 |
| | 6 months | 0.97[a] | 1.09 | 0.88[a] | 0.99 | 0.022 | 0.013 | 0.005 |
| | 7 months | 0.84[ab] | 1.08 | 0.71[b] | 1.05 | 0.033 | 0.071 | <0.001 |
| | 8 months | 0.80[b] | 1.08 | 0.62[b] | 0.96 | 0.035 | <0.001 | <0.001 |
| ***p*-value** | | 0.043 | 0.250 | <0.001 | 0.470 | | | |
| C16 | 5 months | 32.53 | 36.68 | 30.53[ab] | 32.90[b] | 0.524 | <0.001 | <0.001 |
| | 6 months | 33.27 | 35.58 | 32.16[a] | 34.84[ab] | 0.411 | 0.199 | 0.001 |
| | 7 months | 32.96 | 36.27 | 31.42[a] | 36.42[a] | 0.486 | 0.277 | <0.001 |
| | 8 months | 31.62 | 36.42 | 29.05[b] | 34.39[ab] | 0.582 | 0.001 | <0.001 |
| ***p*-value** | | 0.494 | 0.542 | 0.002 | 0.023 | | | |
| C17 | 5 months | 0.45 | 0.38 | 0.51 | 0.47 | 0.018 | 0.024 | 0.090 |
| | 6 months | 0.43 | 0.39 | 0.44 | 0.45 | 0.025 | 0.509 | 0.813 |
| | 7 months | 0.47 | 0.45 | 0.44 | 0.39 | 0.011 | 0.029 | 0.057 |
| | 8 months | 0.43 | 0.49 | 0.43 | 0.32 | 0.021 | 0.031 | 0.469 |
| ***p*-value** | | 0.822 | 0.139 | 0.263 | 0.096 | | | |
| C18 | 5 months | 18.83[c] | 13.12 | 21.33 | 14.64[a] | 0.696 | 0.017 | <0.001 |
| | 6 months | 19.20[bc] | 12.44 | 18.09 | 13.45[ab] | 0.625 | 0.946 | <0.001 |
| | 7 months | 21.35[a] | 12.36 | 19.45 | 12.00[b] | 0.839 | 0.168 | <0.001 |
| | 8 months | 20.92[ab] | 12.50 | 21.05 | 12.86[ab] | 0.807 | 0.709 | <0.001 |
| ***p*-value** | | 0.029 | 0.685 | 0.132 | 0.045 | | | |
| C24 | 5 months | 0.67 | 0.57[c] | 0.50 | 0.50[b] | 0.045 | 0.205 | 0.608 |
| | 6 months | 0.31 | 0.96[bc] | 0.76 | 0.88[ab] | 0.076 | 0.156 | 0.006 |
| | 7 months | 0.53 | 1.25[ab] | 0.66 | 1.01[a] | 0.090 | 0.734 | 0.002 |
| | 8 months | 0.72 | 1.50[a] | 0.62 | 1.14[a] | 0.089 | 0.088 | <0.001 |
| ***p*-value** | | 0.052 | 0.001 | 0.378 | 0.022 | | | |
| ΣSFA | 5 months | 53.45[b] | 52.01 | 53.73 | 49.52 | 0.537 | 0.248 | 0.005 |
| | 6 months | 54.18[ab] | 50.47 | 52.34 | 50.61 | 0.536 | 0.388 | 0.009 |
| | 7 months | 56.15[a] | 51.40 | 52.69 | 50.87 | 0.573 | 0.039 | 0.001 |
| | 8 months | 54.49[ab] | 51.99 | 51.77 | 49.67 | 0.562 | 0.017 | 0.028 |
| ***p*-value** | | 0.148 | 0.324 | 0.657 | 0.798 | | | |

Abbreviations: SFA: saturated fatty acids; SEM: standard error of the mean; values with different superscript letters (a, b, c) within the same column per fixed effect (age of slaughter) differ significantly ($p < 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

**Table S9.** Monounsaturated fatty acid profile of thigh meat at different slaughter ages (g/100g of total fatty acids) (n = 8).

| Items | Age at slaughter | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | | Breed | Gender |
| C16 :1n7 | 5 months | 3.07[a] | 4.67 | 2.53[ab] | 3.88 | 0.196 | 0.022 | <0.001 |
| | 6 months | 2.95[a] | 4.46 | 3.27[a] | 4.24 | 0.174 | 0.865 | <0.001 |
| | 7 months | 2.20[b] | 4.24 | 2.53[ab] | 4.63 | 0.223 | 0.159 | <0.001 |
| | 8 months | 2.47[ab] | 4.61 | 1.97[b] | 3.98 | 0.225 | 0.026 | <0.001 |
| ***p*-value** | | 0.019 | 0.762 | 0.026 | 0.142 | | | |
| C18 :1c9 | 5 months | 34.84 | 36.63 | 32.62[b] | 37.73 | 0.509 | 0.482 | <0.001 |
| | 6 months | 36.44 | 38.98 | 37.41[a] | 40.04 | 0.511 | 0.288 | 0.011 |
| | 7 months | 33.68 | 39.25 | 36.96[a] | 40.25 | 0.602 | 0.016 | <0.001 |
| | 8 months | 34.97 | 37.88 | 34.57[ab] | 40.18 | 0.575 | 0.269 | <0.001 |
| ***p*-value** | | 0.102 | 0.087 | 0.008 | 0.137 | | | |
| C20: 1 | 5 months | 0.40 | 0.39 | 0.53 | 0.55[a] | 0.024 | 0.002 | 0.849 |
| | 6 months | 0.46 | 0.38 | 0.55 | 0.41[b] | 0.023 | 0.153 | 0.011 |
| | 7 months | 0.37 | 0.36 | 0.42 | 0.34[bc] | 0.034 | 0.840 | 0.569 |
| | 8 months | 0.38 | 0.26 | 0.48 | 0.26[c] | 0.036 | 0.466 | 0.021 |
| ***p*-value** | | 0.686 | 0.344 | 0.548 | <0.001 | | | |
| C24:1 | 5 months | 0.02[b] | 0.14 | 0.30 | 0.14 | 0.034 | 0.032 | 0.705 |
| | 6 months | 0.37[a] | 0.25 | 0.18 | 0.19 | 0.041 | 0.138 | 0.468 |
| | 7 months | 0.11[b] | 0.23 | 0.16 | 0.13 | 0.033 | 0.696 | 0.568 |
| | 8 months | 0.22[ab] | 0.31 | 0.13 | 0.14 | 0.036 | 0.065 | 0.479 |
| ***p*-value** | | 0.014 | 0.442 | 0.371 | 0.899 | | | |
| ΣMUFA | 5 months | 38.34[ab] | 41.83 | 35.98[c] | 42.31 | 0.643 | 0.321 | <0.001 |
| | 6 months | 40.22[a] | 44.07 | 41.42[a] | 44.88 | 0.647 | 0.394 | 0.004 |
| | 7 months | 36.36[b] | 44.08 | 40.07[ab] | 45.35 | 0.774 | 0.013 | <0.001 |
| | 8 months | 38.04[ab] | 43.07 | 37.15[bc] | 44.56 | 0.759 | 0.777 | <0.001 |
| ***p*-value** | | 0.049 | 0.230 | 0.020 | 0.148 | | | |

Abbreviations: MUFA: monounsaturated fatty acids; SEM: standard error of the mean; values with different superscript letters (a, b, c) within the same column per fixed effect (age of slaughter) differ significantly ($p < 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

**Table S10.** Polyunsaturated fatty acid profile of thigh meat at different slaughter ages (g/100g of total fatty acids) (n = 8).

| Items | Age at slaughter | *Bionda Piemontese* | | *Bianca di Saluzzo* | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | | Breed | Gender |
| C18 :2n6 | 5 months | 4.59 | 2.98[a] | 4.92 | 3.07[a] | 0.316 | 0.716 | 0.006 |
| | 6 months | 4.10 | 2.13[b] | 3.27 | 2.16[b] | 0.225 | 0.275 | <0.001 |
| | 7 months | 4.12 | 1.72[b] | 4.21 | 1.35[c] | 0.280 | 0.645 | <0.001 |
| | 8 months | 4.69 | 2.03[b] | 6.48 | 2.11[b] | 0.495 | 0.232 | <0.001 |
| ***p*-value** | | 0.719 | 0.001 | 0.110 | <0.001 | | | |
| C20 :2n6 | 5 months | 0.03 | 0.02[a] | 0.02 | 0.02 | 0.007 | 0.781 | 0.968 |
| | 6 months | 0.04 | 0.00[b] | 0.01 | 0.00 | 0.006 | 0.236 | 0.065 |
| | 7 months | 0.00 | 0.00[b] | 0.01 | 0.00 | 0.002 | 0.165 | 0.165 |
| | 8 months | 0.03 | 0.00[b] | 0.04 | 0.01 | 0.010 | 0.755 | 0.112 |
| ***p*-value** | | 0.595 | 0.004 | 0.438 | 0.290 | | | |
| C20: 4n6 | 5 months | 0.46 | 0.49[a] | 1.80[a] | 1.13 | 0.210 | 0.017 | 0.427 |
| | 6 months | 0.43 | 0.13[b] | 0.44[b] | 0.18 | 0.046 | 0.719 | 0.002 |
| | 7 months | 0.49 | 0.12[b] | 0.45[b] | 0.07 | 0.058 | 0.655 | 0.001 |
| | 8 months | 0.66 | 0.11[b] | 0.37[b] | 0.45 | 0.089 | 0.882 | 0.168 |
| ***p*-value** | | 0.702 | 0.041 | 0.003 | 0.063 | | | |
| Σn6 PUFA | 5 months | 5.08 | 3.49[a] | 6.73[a] | 4.23[a] | 0.358 | 0.056 | 0.002 |
| | 6 months | 4.57 | 2.26[b] | 3.72[b] | 2.35[b] | 0.271 | 0.389 | <0.001 |
| | 7 months | 4.61 | 1.84[b] | 4.67[ab] | 1.42[c] | 0.323 | 0.624 | <0.001 |
| | 8 months | 5.39 | 2.14[b] | 6.90[a] | 2.56[b] | 0.502 | 0.207 | <0.001 |
| ***p*-value** | | 0.674 | 0.001 | 0.045 | <0.001 | | | |
| C18 :3n3 | 5 months | 0.08 | 0.09 | 0.22 | 0.15 | 0.025 | 0.057 | 0.514 |
| | 6 months | 0.19 | 0.08 | 0.13 | 0.10 | 0.025 | 0.782 | 0.188 |
| | 7 months | 0.17 | 0.12 | 0.12 | 0.06 | 0.024 | 0.293 | 0.240 |
| | 8 months | 0.13 | 0.09 | 0.05 | 0.03 | 0.018 | 0.061 | 0.468 |
| ***p*-value** | | 0.652 | 0.800 | 0.170 | 0.104 | | | |
| C20 :5n3 | 5 months | 0.00 | 0.09 | 0.00 | 0.07 | 0.022 | 0.793 | 0.079 |
| | 6 months | 0.00 | 0.29 | 0.07 | 0.06 | 0.064 | 0.542 | 0.281 |
| | 7 months | 0.00 | 0.13 | 0.00 | 0.05 | 0.026 | 0.484 | 0.085 |
| | 8 months | 0.11 | 0.27 | 0.00 | 0.01 | 0.056 | 0.102 | 0.461 |
| ***p*-value** | | 0.407 | 0.757 | 0.407 | 0.693 | | | |
| C22 :5n3 | 5 months | 0.00 | 0.00 | 0.04[b] | 0.15 | 0.028 | 0.103 | 0.335 |
| | 6 months | 0.00 | 0.11 | 0.00[b] | 0.00 | 0.029 | 0.326 | 0.326 |
| | 7 months | 0.00 | 0.00 | 0.00[b] | 0.00 | - | - | - |
| | 8 months | 0.00 | 0.00 | 0.62[a] | 0.00 | 0.097 | 0.094 | 0.094 |
| ***p*-value** | | - | 0.407 | 0.055 | 0.132 | | | |
| C22 :6n3 | 5 months | 0.24[a] | 0.00 | 0.00[b] | 0.12 | 0.041 | 0.441 | 0.441 |
| | 6 months | 0.00[b] | 0.12 | 0.00[b] | 0.00 | 0.030 | 0.326 | 0.326 |
| | 7 months | 0.00[b] | 0.00 | 0.00[b] | 0.00 | - | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 8 months | $0.00^{b}$ | 0.00 | $0.94^{a}$ | 0.00 | 0.146 | 0.090 | 0.090 |
| ***p*-value** | | 0.028 | 0.407 | 0.043 | 0.132 | | | |
| Σn3 PUFA | 5 months | 0.32 | 0.19 | 0.25 | $0.51^{a}$ | 0.059 | 0.263 | 0.631 |
| | 6 months | 0.19 | 0.60 | 0.20 | $0.16^{b}$ | 0.081 | 0.187 | 0.238 |
| | 7 months | 0.17 | 0.25 | 0.12 | $0.12^{b}$ | 0.037 | 0.257 | 0.667 |
| | 8 months | 0.24 | 0.36 | 1.60 | $0.04^{b}$ | 0.242 | 0.262 | 0.126 |
| ***p*-value** | | 0.753 | 0.369 | 0.078 | 0.011 | | | |
| ΣPUFA | 5 months | 5.40 | $3.67^{a}$ | $6.99^{ab}$ | $4.74^{a}$ | 0.379 | 0.053 | 0.005 |
| | 6 months | 4.76 | $2.87^{b}$ | $3.93^{c}$ | $2.51^{bc}$ | 0.267 | 0.201 | 0.001 |
| | 7 months | 4.79 | $2.09^{b}$ | $4.80^{bc}$ | $1.54^{c}$ | 0.319 | 0.458 | <0.001 |
| | 8 months | 5.63 | $2.50^{b}$ | $8.50^{a}$ | $2.60^{b}$ | 0.564 | 0.052 | <0.001 |
| ***p*-value** | | 0.623 | 0.002 | 0.004 | <0.001 | | | |

Abbreviations: PUFA: polyunsaturated fatty acids; SEM: standard error of the mean; values with different superscript letters (a, b, c) within the same column per fixed effect (age of slaughter) differ significantly ($p < 0.05$). The effect of interaction between "Breed" and "Gender" was not significant; therefore, significance is only presented for main effects.

## 9. Black soldier fly larvae used for environmental enrichment purposes: Can they affect the growth, slaughter performance, and blood chemistry of medium-growing chickens?

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 14 December 2022
DOI 10.3389/fvets.2022.1064017

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# Black soldier fly larvae used for environmental enrichment purposes: Can they affect the growth, slaughter performance, and blood chemistry of medium-growing chickens?

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SPECIALTY SECTION
This article was submitted to Animal Nutrition and Metabolism, a section of the journal Frontiers in Veterinary Science

RECEIVED 07 October 2022
ACCEPTED 23 November 2022
PUBLISHED 14 December 2022

CITATION
Bongiorno V, Gariglio M, Zambotto V, Cappone EE, Biasato I, Renna M, Forte C, Coudron C, Bergagna S, Gai F and Schiavone A (2022) Black soldier fly larvae used for environmental enrichment purposes: Can they affect the growth, slaughter performance, and blood chemistry of medium-growing chickens? *Front. Vet. Sci.* 9:1064017. doi: 10.3389/fvets.2022.1064017



**Introduction:** This research has been aimed at evaluating the effects of live black soldier fly larvae (BSFL) (*Hermetia illucens*) on the growth, slaughtering performance, and blood parameters of medium-growing chickens.

**Methods:** A total of 240, 28-day-old, Label Rouge Naked Neck chickens were allotted to four experimental groups, according to the gender (males-females) and to the absence (control group, C) or presence (larvae group, L) of a dietary supplementation with 10% live BSFL, on the basis of the expected average daily feed intake (ADFI) (6 replicates/diet, 10 chickens/replicate). The birds were weighed weekly, and the feed consumption was recorded to calculate the average live weight, feed conversion ratio (FCR), average daily gain (ADG), and the ADFI. At 82 days of age, 2 birds/replicate (12 birds/diet) were selected and slaughtered. The blood samples were collected, and the carcass traits (carcass, breast, thigh, and organ weights and yields) were assessed.

**Results and discussions:** Overall, the administered live BSFL did not impair the growth and slaughtering performance, or the blood traits, while the C females showed a better FCR than the treated ones ($P < 0.05$). The live BSFL consumption time was longer for the females than for the males ($P < 0.001$). The weight of the immune organs (spleen and bursa of Fabricius) increased as the live BSFL supplementation increased ($P < 0.05$). Furthermore, the provision of live BSFL reduced the gamma glutamyl transferase (GGT, U/l) activity content in the blood ($P < 0.05$). Finally, both the leukocytes (%) and the monocytes (%) were more abundant in the C groups than in the larvae ones ($P < 0.05$ and $P < 0.01$, respectively). In short, the supplementation of live BSFL can be used successfully as an environmental enrichment, without affecting the growth performance of male birds. Furthermore, the immune organ activity could be enhanced by the provision of live BSFL.

KEYWORDS

**organic rearing, live larvae, free-range chickens, larva consumption duration, animal welfare**

## Introduction

It is well known that organic and free-range rearing systems for poultry are perceived by consumers to provide sustainable products, thereby guaranteeing environmental protection and animal welfare (1). These aspects also represent fundamental factors that can affect the choices of consumers and can orient their purchasing decisions (2–4). Benefits can hence be introduced for the farmer who, by guaranteeing an increase in animal welfare, can obtain significant economic profits. It has been demonstrated that the welfare status of broilers can influence their performance (5). The use of insects in chicken diets could be a valid tool, because of their characteristics, to support and promote bird welfare. Indeed, live insects can be considered as an environmental enrichment, as they are able to stimulate the curiosity of poultry as a result of their motility (6). Moreover, it is known that the quality of the substrate in terms of visual, tactile and gustatory appreciation, affects the interest of birds. Hence, the larvae's abundance in moisture and fat could be an interesting feed source for poultry, useful to balance their nutrient intake (6, 7). Biasato et al. (8) have recently reported an improvement in the welfare of broilers fed diets supplemented with 5% yellow mealworm (*Tenebrio molitor*) or 5% live black soldier fly larvae (*Hermetia illucens*) (BSFL), as well as an increase in physical activity. Furthermore, insects are a natural feed source for poultry, and are capable of promoting their foraging behavior, as they capture their attention and reduce the poultry aggressiveness toward conspecifics (6, 9). Finally, insects can minimize and valorize food waste, which is a promising substrate for insect feeding (6, 10–12). Nevertheless, the provision of live larvae to poultry has been poorly investigated. The few published papers about the use of live insect larvae in poultry nutrition have mainly focused on the provision of live black soldier fly larvae to turkeys (13), broiler chickens (14, 15), and laying hens (16, 17), and have evaluated the effects on the growth, health status, and slaughtering performance of the birds. Veldkamp and van Niekerk (13) revealed a higher daily feed intake (DFI) and body weight gain in turkeys fed 10% live BSFL (on an as fed basis) than control groups. The physical exertion of broilers, as well as their hock burn score, can be improved by the administration of live larvae (supplemented as fed of 5 or 10% live BSFL, twice or four times/day), as reported by Ipema et al. (14). Dietary supplementation with live yellow mealworm (5% as fed) further improved the feed conversion ratio (FCR) of broilers in a trial conducted by Bellezza Oddon et al. (15). Finally, a lower feed consumption and a higher live body weight increase were reported in laying hens fed live BSFL *ad libitum* than in control birds and groups with controlled larvae provision (10% or 20% as fed) (17). In addition to these parameters, the recording of the larvae consumption duration could be useful, and this method had already been experimented by Veldkamp and van Niekerk (13) and Bellezza Oddon et al. (15) to evaluate the appreciation of larvae by birds, as well as to monitor their level of confidence in larvae consumption over time. Moreover, the effect of insect provision could be reflected in the blood traits, which is an important animal welfare indicator (18). A previous study reported no negative effects on blood traits of broiler chickens supplemented with live BSFL and yellow mealworm larvae (15), while a reduction in triglycerides and in the cholesterol levels was observed in the blood of Muscovy ducks fed increasing levels (0, 3, 6, and 9%) of black soldier fly meal (16).

The aim of this research was to provide novel insights into the effects of the provision of live BSFL in free-range and organic systems, since chickens intended for meat consumption have never been tested before from a long-term rearing perspective (considering the classic broiler rearing cycle). Thus, the growth and slaughtering performance, the larvae consumption duration, and the blood parameters of the birds were investigated in relation to this kind of supplementation.

## Materials and methods

### Animals and husbandry

A total of 300 1-day-old Label Naked Neck birds (Hubbard JA57 hybrid) were purchased from a commercial hatchery (sexed chicks, sex ratio 1:1) and transferred to the poultry facility of the University of Turin (Department of Agricultural, Forest and Food Sciences) (North-West Italy), where the trial was carried out (coordinates latitude: 44.88572, longitude: 7.68381, altitude: 239 mamsl). The experiment was previously approved by the Bioethical Committee of the University of Turin (Italy; Prot. No. 814715). The birds were vaccinated against coccidiosis, and Newcastle and Marek's diseases. The chicks were reared from 1 to 21 days of age in an environmentally controlled poultry house and distributed according to gender in four 3.4 m × 2.9 m pens with rice hulls as bedding. Each pen was equipped with 3 feeders and 3 drinkers, and automatic ventilation and illumination systems were available. The adopted lighting schedule was 23L:1D during the first 3 days, according to the Hubbard guidelines (19). Moreover, infrared lamps were at the disposal of the birds for the first 2 weeks of age. Subsequently, the photoperiod was gradually modulated to recreate, as much as possible, the natural environmental conditions of the experimental building in which the birds were to be housed at 21 days of age (12L:12D). At that moment,

Abbreviations: BSFL, black soldier fly larvae; CF, control females; CM, control males; LF, larvae females; LM, larvae males; M, males; F, females; LW, live weight; ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; SW, slaughter weight; GGT, gamma glutamyl transferase; CC, chilled carcass; RTCC, ready to cook carcass.

the birds were individually weighed and tagged with a wing mark. Then 120 males (M) and 120 females (F) were selected, on a uniform live weight (LW, g) basis, and allotted to 24 pens. The 2.2 × 3.5 m pens (with rice husk as bedding) each had the possibility of outdoor access (2.2 × 4.5 m), which was ensured for all the birds from 49 days of age to the end of the experiment (82 days of age). All the birds were subjected to the same management and environmental conditions in respect of the European Union's regulations on organic farming (20). Natural ventilation and photoperiods (from 12L:12D in October 2021 to 10L:14D at the end of November 2021) were applied for the entire duration of the trial. The average temperature was 12.8°C (min 5°C; max 22°C) in October 2021 and 7.6°C (min −1°C; max 16°C) in November 2021. The mortality and health status of the birds were checked and recorded daily. Regarding the experimental design, four experimental groups were created on d 21 according to the gender and the diet (10 chicken/pen, 6 replicates/diet):

1. Control males (CM): fed a basal organic feed.
2. Control females (CF): fed a basal organic feed.
3. Larvae males (LM): fed a basal organic feed, supplemented with 10% live BSFL (as fed, 33.63/100g on dry matter–DM) on the basis of the expected DFI.
4. Larvae females (LF): fed a basal organic feed, supplemented with 10% live BSFL (as fed, 33.63/100g DM) on the basis of the expected DFI.

The expected DFI of the birds was taken from the Hubbard guidelines (19). The birds always had free access to water and feeds. A starter diet was adopted until d 28 (22.92/100g of crude protein (CP), 15.36 MJ/kg of apparent metabolizable energy) and a grower feed was then provided from d 28 to d 82 (20.55/100g of crude protein, 14.19 MJ/kg of apparent metabolizable energy) (Verzuolo Biomangimi s.r.l.—Verzuolo, CN, Italy). The feed was analyzed, and the DM (method number 934.01), ash (method number 942.05), and ether extract (EE, method number 2003.05) were calculated [DM, ash; EE: (21)]. The CP was determined by means of the Dumas method (22, 23). The feed composition of the feeds is reported in the Table 1. Provision of live larvae was started on d 28, after one-week of adaptation of the birds to the new environment.

TABLE 1 Ingredients and the analyzed chemical composition of the experimental diets[a].

| Ingredients | Starter period (1–28 days) | Grower period (28–81 days) |
|---|---|---|
| Corn meal | 43.00 | 50.48 |
| Soybean meal | 28.60 | 16.26 |
| Sunflower meal | 10.00 | 14.46 |
| Corn gluten meal | 3.50 | 4.70 |
| Pea beans | 7.00 | 8.00 |
| Alfalfa meal | 2.00 | 2.50 |
| Soybean oil | 1.00 | - |
| Dicalcium phosphate | 1.70 | 0.85 |
| Calcium carbonate | 1.55 | 1.25 |
| Sodium bicarbonate | 0.10 | - |
| Mineral-vitamin premix[b] | 1.50 | 1.50 |
| PoultryStar®[c] | 0.05 | - |
| Chemical composition (g/100 g on an as fed basis) | | |
| DM | 90.44 | 90.61 |
| CP | 22.92 | 20.55 |
| EE | 6.19 | 5.02 |
| CF | 5.85 | 6.26 |
| Ash | 7.81 | 5.65 |
| AMEn[d], MJ/kg | 15.36 | 14.19 |
| Mineral composition | | |
| Ca | 1.27 | 0.96 |
| P | 0.64 | 0.49 |
| Na | 0.18 | 0.15 |
| Aminoacids | | |
| Methionine | 0.34 | 0.33 |
| Lysine | 1.04 | 0.83 |

DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; AME, gross energy.

[a] Values are reported as the mean value of duplicated analyses.

[b] Nutritional additives: Vitamin A 8.001.60 UI, Vitamin D3 3.000.60 UI, Betaine anhydrous 600.48 mg, Biotin 0.04 mg, Choline chloride 333.07 mg, Folic acid 0.81 mg, Niacinamide 25.01 mg, Calcium pantothenate 7.28 mg, Vitamin B1 0.75 mg, Vitamin B12 0.02 mg, Vitamin B6 1.60 mg, Vitamin E 18.50 mg, Vitamin K3 2.50 mg, Copper (Copper-II sulfate pentahydrate) 10.00 mg, Iodine (Calcium iodate anhydrous) 1.50 mg, Iron (Iron-II sulfate monohydrate) 44.01 mg, Manganese (Manganese-II oxide) 62.01 mg, Selenium (Sodium Selenite) 0.25 mg, Zinc (Zinc sulfate monohydrate) 50.01 mg. Zootechnical additives: 4a1604i Endo-1,3(4)-beta-glucanase EC 3.2.1.6 1.500,30 UV, 4a1604i Endo-1,4-beta-xylanase EC 3.2.1.8 1.100,22 UV, Technological additives: E 562 Sepiolite 224.32 mg, 1m558 Bentonite 0.54 mg.

[c] Poultrystar®: prebiotic and probiotic complex produced by Koninklijke DSM N.V., Heerlen, The Netherlands.

[d] Calculated according to INRA (24).

## Larvae management and chemical analyses

The live BSFL were provided by INAGRO (Ieperseweg 87, 8800 Rumbeke-Beitem–Belgium). The larvae were shipped weekly in an insulated container with cool bags to keep them chilled and to avoid their death during the 24-h journey. Once the larvae had arrived at the poultry experimental facility, they were stored at 16°C in a climatic chamber to induce the diapause of insects and fix the larvae instar for the entire week (25). A total of 100 g of BSFL samples were collected each week and stored at −80°C and subsequently analyzed for DM (method number 934.01), ash (method number 942.05), and EE (method number 2003.05) [DM, ash; EE: (21)]. The CP and the chitin contents were determined by means of the Dumas and Finke methods, respectively (22, 23, 26). The gross energy (GE) content was

TABLE 2 The analyzed proximate and amino acid composition of black soldier fly larvae.

| Proximate composition, g/100 g on an as fed basis | Values[a] |
|---|---|
| DM | 33.63 |
| CP | 14.39 |
| EE | 9.56 |
| Ash | 4.34 |
| Chitin | 2.00 |
| GE, MJ/kg | 8.69 |
| Amino acid, g/100 g on an as fed basis | |
| Alanine | 1.01 |
| Arginine | 0.71 |
| Aspartic acid | 1.28 |
| Cysteine | 0.17 |
| Glutamic acid | 1.65 |
| Glycine | 0.77 |
| Hystidine | 0.44 |
| Isoleucine | 0.61 |
| Leucine | 0.94 |
| Lysine | 0.94 |
| Methionine | 0.27 |
| Phenylalanine | 0.57 |
| Proline | 0.77 |
| Serine | 0.64 |
| Threonine | 0.54 |
| Tryptophane | 2.02 |
| Tyrosine | 0.87 |
| Valine | 0.81 |

DM, dry matter; CP, crude protein; EE, ether extract; GE, gross energy.
[a]Values are reported as the mean value of duplicated analyses.

obtained by means of an adiabatic calorimetric bomb (C000; IKA). Finally, an analysis of the aminoacidic (AA) composition was performed according to the method used by Hewitson et al. (27). The average composition of the BSFL is reported in the Table 2.

Prior to being administered to the chickens, the live BSFL were reactivated by heating them at 28°- 30°C for 10 min, in accordance with what was reported by Bellezza Oddon et al. (15). The live BSFL were provided daily at 11.00 a.m., except on Sundays. The larvae consumption duration was recorded daily using a stopwatch that started when a plate was placed in the pen and ended when the plate was empty. The duration of the BSLF consumption was monitored throughout the experimental period considering five recording periods, composed of 10 days each, except for the last one, which only lasted 7 days. The five-recording periods were defined as follows: T1: 28–39 d, T2: 40–50 d, T3: 51–62 d, T4: 63–74 d, and T5: 75–81 d. The larvae were distributed in each pen on two plates (18.8 linear cm and 141.3 $cm^2$/chick, Ø 30 cm). Two empty plates were also introduced into the C bird pens.

## Growth performance

The LW (g) and the feed consumption (g) were recorded weekly (electronic scales; KERN PLE-N v. 2.2; KERN & Sohn GmbH; d: 0.1) on a pen basis for the whole experimental period (28–81 d). The average daily gain (ADG, g/d), the average daily feed intake (ADFI, g/d), and the FCR (g/g, DM basis) were calculated both on a weekly basis and for the overall period (28–81 d) on a pen basis. The ADFI was calculated on an as fed basis without considering the larvae administration. However, the average amount of provided BSFL (on an as fed basis, g) was determined. Finally, the DM of the larvae was analyzed (33.63/100 g) and used to adjust the FCR of the birds, according to the method used by Veldkamp and van Niekerk (13) and by Bellezza Oddon et al. (15).

## Slaughtering performance

A total of 48 birds (two birds/pen, 12 birds/diet) were selected on d 82 on the basis of the average LW of the pen and labeled with a shank ring. The individual slaughtering weight (SW, g) was recorded. The birds were slaughtered in a commercial abattoir and blood samples were collected during bleeding. The weight of the ready-to-cook carcass (RTCC, g) (plucked carcasses without feet, neck, head, and organs) was registered. The absolute weight of the heart, spleen, bursa of Fabricius, liver, and abdominal fat was recorded, and the relative weights were calculated as a percentage of the SW. After 24 h of refrigeration (4°C), the chilled carcass (CC, g) weight was registered, and the weight reported as a % of SW. The RTCC yield (%SW), CC yield (%SW), breast yields (%CC weight) and thigh yields (%CC weight) were also calculated.

## Blood analyses

Blood samples were collected from the jugular vein of the 48 selected birds at slaughtering. A total of 2.5 mL was placed in a $K_3$EDTA tube and 2.5 mL in a serum-separating tube to evaluate the hematological parameters. A blood smear was prepared from a drop of blood without anticoagulant. A glass slide was used for each bird. The smears were stained using May-Grünwald and Giemsa stains. The total red (erythrocytes, $10^6$, cell/μL) and white (leukocytes, $10^3$, cell/μL) blood cell counts were determined in an improved Neubauer hemocytometer on blood samples previously treated with a 1:200 Natt-Herrick solution (28). One hundred leukocytes, including granular (heterophils, eosinophils, and basophils) and non-granular (lymphocytes

TABLE 3 The growth performance of male and female Label Rouge Naked Neck birds fed a diet supplemented with 10% live black soldier fly larvae; supplementation based on the expected daily feed intake (28–81 d; $n = 6$).

| Item | Days | Diet (D) | | Gender (G) | | SEM | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BSFL | Control | Male | Female | D | G | D x G | D | G | D x G |
| LW, g | 28 | 479 | 475 | 515 | 439 | 2.07 | 2.07 | 2.92 | 0.186 | <0.001 | 0.298 |
| | 81 | 2,372 | 2,340 | 2,742 | 1,970 | 11.40 | 11.40 | 16.12 | 0.047 | <0.001 | 0.281 |
| ADG, g/d | 28–81 | 35.6 | 35.2 | 41.8 | 28.9 | 0.24 | 0.24 | 0.34 | 0.229 | <0.001 | 0.700 |
| ADFI, g/d | 28–81 | 111 + 9.93* | 112 | 125 | 98.6 | 1.16 | 1.16 | 1.65 | 0.572 | <0.001 | 0.152 |
| FCR, g/g | 28–81 | 2.93 | 2.92 | 2.74 | 3.11 | 0.02 | 0.02 | 0.03 | 0.722 | <0.001 | 0.002 |

LW, live weight; ADG, average daily gain; ADFI, average daily feed intake (on an as fed basis).
*Indicates the average amount of larvae provided (on an as fed basis, average DM: 33.63/100 g).
FCR, feed conversion ratio (on a dry matter basis, including the larvae intake); BSFL, black soldier fly larvae; SEM, standard error of the mean.

and monocytes) leukocytes, were counted on the slide and expressed as a percentage of the total leukocytes, according to Campbell (29). Samples from tubes without any anticoagulant had previously been centrifuged at 3,500 rpm for 15 min at 20°C, after having been left at room temperature for 2 h to favor blood clot formation. The concentrations of alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), creatinine (CRE, mg/dL), total proteins (g/dL), uric acid (mg/dL), cholesterol (mg/dL), triglycerides (mg/dL), gamma glutamyl transferase (GGT, U/L), phosphorus (P, mg/dL), iron (Fe, µg/dL), and magnesium (Mg, mg/dL) were measured using a compact liquid chemistry analyzer system (BT 1500 vet–Futurlab, Padua, Italy).

## Statistical analysis

The data were analyzed by means of IBM SPSS Statistics software, V20.0.0 (IBM). The homogeneity of variance was established by means of Levene's test, and the normality or non-normality of residuals was assessed by means of Shapiro-Wilk's test. The pen was considered as the experimental unit for the larvae consumption duration and growth performance (n=6 per diet), while the animal was used as the experimental unit for the slaughtering performance and the blood parameters (n=12 per diet). A general linear mixed model (GLMM), with a gamma probability distribution and log-link function, was performed to analyze the larvae consumption duration, where the time (i.e., the above mentioned 5 recording periods, namely T1–T5), gender and their interaction were considered as fixed effects, as evaluated by pairwise comparisons, and the replicate was included as repeated measurements on the same pen. A general linear model (GLM) was used for the growth and slaughtering performances and for the blood analyses. The gender, diet, and the interaction between gender and diet were considered by means of a pairwise comparison. $P \leq 0.05$ were declared as statistically significant, while a statistical trend was defined for $P \leq 0.10$.

# Results

## Growth performance

The health status of the birds was checked daily, and no mortality was recorded throughout the whole experiment. The growth performance of the birds is summarized in Table 3. A statistical analysis, based on the weekly measurements, was performed, and the complete table is reported in the Supplementary Table S1.

Overall, the larvae supplementation did not affect the growth performance of the birds, apart from the LW at 81 days of age, when the treated birds were heavier (+32.12 g) than the C ones ($P < 0.05$). Instead, the gender always had a significant effect on the growth performance parameters. In other words, the M displayed a higher LW, ADG, and ADFI than the F, as well as a better FCR ($P < 0.001$) (Table 3). The interaction between the gender and the diet only affected the FCR ($P < 0.05$), that is, the LF showed a higher FCR than the CF and the LM ($P < 0.05$ and $P < 0.001$, respectively). Finally, the LM tended to display a lower FCR than the CM ($P = 0.058$) (Figure 1). Regarding the weekly measurements, the treated groups displayed a higher LW than the controls from 42 days of age onward ($P < 0.05$), even if no significant differences were observed at 70 d of age (Figure 2). Moreover, improvements in the treated groups, compared to the controls, were recorded during the first 2 weeks of the trial (ADFI at 28–35 d and 35–42 d, $P < 0.01$ and $P = 0.01$, respectively; ADG and FCR at 35–42 d, $P < 0.05$; FCR at 28–35 d, $P < 0.001$, Supplementary Table S1). Further advantages were observed in the LM (ADG and FCR at 28–35 d, $P < 0.05$ and $P < 0.001$, respectively (Supplementary Figures S1, S2).

## Larvae consumption duration

The difference between males and females in the larvae consumption duration for each of the five recording periods is

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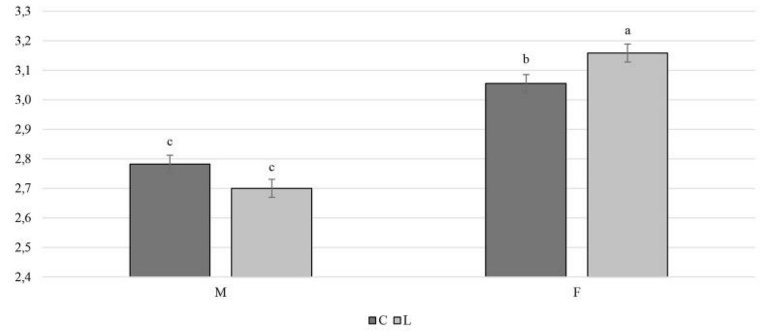


FIGURE 1
Interaction effect (gender×diet) on the feed conversion ratio of the Label Rouge Naked Neck birds fed a diet supplemented with 10% live black soldier fly larvae; supplementation based on the expected daily feed intake, (28–81 d; $n = 6$). a, b indicate significant differences at $P < 0.05$; F, females; M, males; C, control; L, larvae.

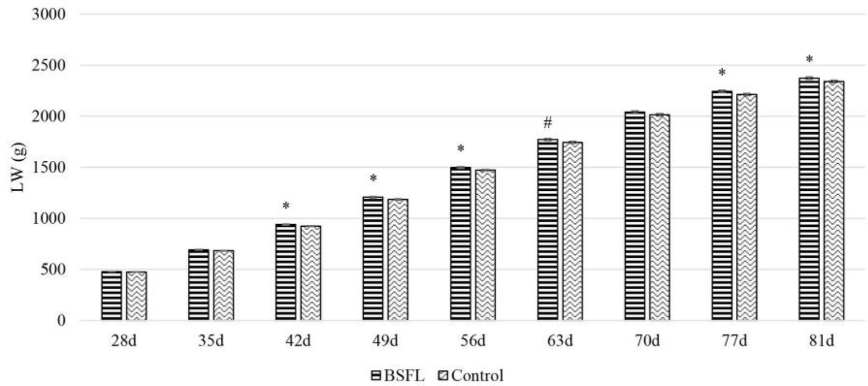


FIGURE 2
The growth curves of the Label Rouge Naked Neck birds fed a diet supplemented with 10% live black soldier fly larvae; supplementation based on the expected daily feed intake, (28–81 d; $n = 6$). # Indicates a statistical trend for the control and BSFL supplemented birds ($P \leq 0.10$); *Indicates a statistical difference between the control and BSFL supplemented birds ($P \leq 0.05$). LW, live weight.

reported in Figure 3. Once the *in vivo* trial had been concluded, the real larvae intake was calculated. The study intended to provide the chickens with 10% live BSFL, on the basis of the expected daily feed intake, as reported by Hubbard (19). However, when the real feed intake was considered, BSFL ingestion was 10.41% and 8.01% for the F and M, respectively. Overall, the gender had a significant effect on the larvae consumption duration (min), which was lower in M than in F (4.97 vs. 10.76 min, $P < 0.01$). Furthermore, the age of the birds also had a significant effect on this parameter. The highest values were recorded at T1 for both sexes (T1:16.52, T2:5.90, T3:4.03, T4:5.29, T4:3.89 min, $P < 0.001$). The interaction between time and gender significantly influenced the larvae consumption duration ($P < 0.01$). In particular, significant differences were recorded between sexes at T3 and T5, while a statistical trend was observed at T4, when the F spent more time than the M on larvae consumption ($P < 0.001$, $P < 0.05$ and $P = 0.072$, respectively) (Figure 3). Moreover, the F ate larvae faster at T5 than at T4 ($P < 0.05$), but no significant differences were recorded between T5 and T2-T3. The M tended to consume the larvae faster at T2 than at T3 ($P = 0.066$), while a significant reduction in larvae consumption duration was observed between T2 and T5

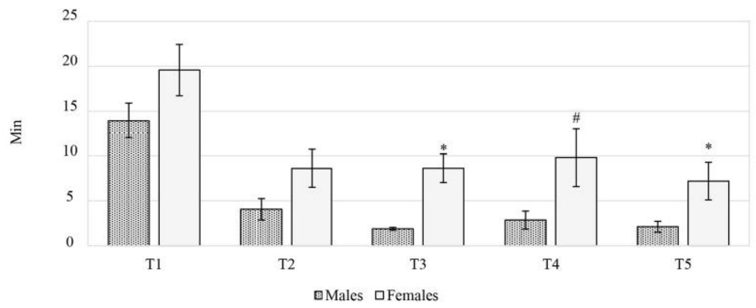


FIGURE 3
The time spent by the Label Rouge Naked Neck birds on eating live black soldier fly larvae ($n = 10$). *$P \leq 0.05$; # $P \leq 0.10$. T1, 28–39 days of age; T2, 40–50 days of age; T3, 51–62 days of age; T4, 63–74 days of age; T5, 75–81 days of age.

**TABLE 4** The slaughtering performance of the male and female Label Rouge Naked Neck birds fed a diet supplemented with 10% live black soldier fly larvae; supplementation based on the expected daily feed intake (28–81d; $n = 12$).

| Item | Diet (D) | | SEM | Gender (G) | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | BSFL | Control | | Male | Female | | D | G | D × G |
| Slaughter weight (g) | 2,441 | 2,423 | 10.00 | 2,829 | 2,035 | 10.00 | 0.206 | <0.001 | 0.161 |
| CC yield (%SW) | 65.2 | 64.7 | 0.28 | 65.3 | 64.6 | 0.28 | 0.213 | 0.091 | 0.193 |
| RTCC yield (%SW) | 66.2 | 65.7 | 0.30 | 66.3 | 65.6 | 0.30 | 0.256 | 0.132 | 0.183 |
| Breast yield (%CC) | 23.2 | 23.2 | 0.21 | 22.2 | 24.2 | 0.21 | 0.815 | <0.001 | 0.894 |
| Thigh yield (%CC) | 33.9 | 34.0 | 0.20 | 34.8 | 33.1 | 0.20 | 0.700 | <0.001 | 0.953 |
| Spleen (%SW) | 0.16 | 0.14 | 0.00 | 0.14 | 0.15 | 0.00 | 0.002 | 0.150 | 1.000 |
| Bursa of Fabricius (%SW) | 0.22 | 0.21 | 0.01 | 0.21 | 0.23 | 0.01 | 0.619 | 0.224 | 0.020 |
| Liver (%SW) | 1.87 | 1.85 | 0.04 | 1.81 | 1.90 | 0.04 | 0.739 | 0.087 | 0.975 |
| Heart (%SW) | 0.51 | 0.48 | 0.01 | 0.52 | 0.47 | 0.01 | 0.057 | 0.001 | 0.771 |
| Abdominal fat (%SW) | 1.43 | 1.60 | 0.18 | 1.01 | 2.02 | 0.18 | 0.511 | <0.001 | 0.270 |

SW, slaughter weight; CC, cold carcass; RTCC, ready to cook carcass; BSFL, black soldier fly larvae; SEM, standard error of the mean.

($P < 0.05$). Finally, the M tended to eat larvae faster at T5 than at T4 ($P = 0.055$) (data not shown).

## Slaughtering performance

The slaughtering performance is reported in Table 4. As expected, the SW (g) was influenced by the gender, with the M being heavier than the F ($P < 0.001$). No differences were observed in the CC yield (%LW) or in the RTCC yield (%LW) between the treated and the C groups, or in the RTCC yield (%LW). A trend was recorded for the gender, with the M tending to display a higher CC yield than the F ($P = 0.091$). The breast yield (%CC weight) was higher in the F than in the M ($P < 0.001$). On the other hand, the M showed a better thigh yield (%CC weight) than the F ($P < 0.001$). As far as the organ weights are concerned, the C groups showed a lower relative weight of the spleen (%SW) than the supplemented ones ($P < 0.01$). A statistical trend of the relative weight of the liver (%SW) was observed for gender and tended to be greater in the F than in the M ($P = 0.087$). The interaction between diet and gender had a significant impact on the relative weight of the bursa of Fabricius (%SW). Specifically, the LF groups displayed a higher relative weight of the bursa of Fabricius than the CF and the LM ones ($P < 0.05$) (Figure 4). The relative weight of the heart (%SW) was higher in the M than in the F ($P = 0.001$). Moreover, the diet tended to affect the relative weight of the heart (%SW), with the C groups which tended to show a lower value than the treated ones ($P = 0.057$). Finally, the relative weight of the abdominal fat (%SW) was greater in the F than in the M ($P < 0.001$) (Table 4).

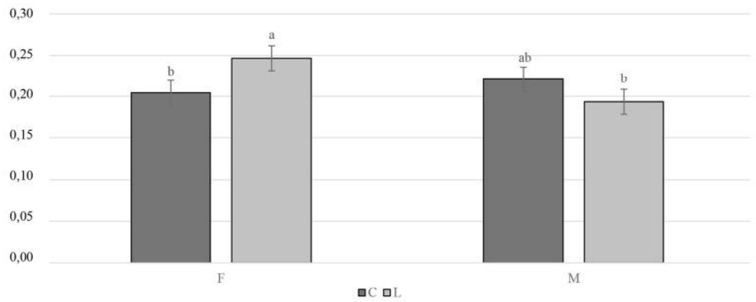


FIGURE 4
Interaction effect (gender × diet) on the bursa of Fabricius relative weight of the Label Rouge Naked Neck birds fed a diet supplemented with 10% live black soldier fly larvae; supplementation based on the expected daily feed intake, (28–81 d; $n = 6$). a, b indicate significant differences at $P < 0.05$; F, females; M, males; C, control; L, larvae.

**TABLE 5** The hematological traits and serum proteins and lipids of the male and female Label Rouge Naked Neck birds fed a diet supplemented with 10% live black soldier fly larvae; supplementation based on the expected daily feed intake (28–81d; $n = 12$).

| Item | Diet (D) | | SEM | Gender (G) | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | BSFL | Control | | Male | Female | | D | G | D x G |
| Erythrocytes, $10^6$, cell/μL | 2.32 | 2.22 | 0.23 | 2.49 | 2.07 | 0.23 | 0.770 | 0.191 | 0.941 |
| Leukocytes, $10^3$, cell/μL | 31.1 | 23.9 | 2.26 | 26.7 | 27.2 | 2.24 | 0.023 | 0.745 | 0.955 |
| Heterophils, % | 47.3 | 42.1 | 2.90 | 47.2 | 42.2 | 2.90 | 0.202 | 0.225 | 0.976 |
| Lymphocytes, % | 48.1 | 52.8 | 2.65 | 48.2 | 52.8 | 2.65 | 0.212 | 0.218 | 0.955 |
| Eosinophils, % | 1.40 | 1.55 | 0.18 | 1.17 | 1.85 | 0.19 | 0.541 | 0.008 | 0.385 |
| Monocytes, % | 1.63 | 3.06 | 3.33 | 2.85 | 1.75 | 0.32 | 0.002 | 0.016 | 0.373 |
| Basophils, % | 2.90 | 2.67 | 0.32 | 2.54 | 3.05 | 0.32 | 0.617 | 0.269 | 0.495 |
| Serum proteins and lipids | | | | | | | | | |
| Total protein, g/dL | 4.61 | 4.73 | 0.11 | 4.70 | 4.65 | 0.11 | 0.405 | 0.743 | 0.747 |
| Cholesterol, mg/dL | 107 | 119 | 5.14 | 116 | 109 | 5.14 | 0.091 | 0.345 | 0.520 |
| Triglycerides, mg/dL | 112 | 107 | 8.54 | 98.9 | 121 | 8.54 | 0.157 | 0.061 | 0.177 |

BSFL, black soldier fly larvae; SEM, standard error of the mean.

## Blood traits

The results about the blood traits of the birds are reported in Tables 5, 6. Overall, the live provision of BSFL did not impair most of the hematological traits or serum proteins and lipids, serum minerals, or liver and renal enzymes. As reported in Table 5, the leukocyte percentage was higher in the treated groups than in the C groups ($P < 0.05$). The eosinophil percentage was higher in the F than in the M ($P < 0.01$), whereas the opposite was found for monocytes ($P < 0.05$). Moreover, the C groups showed a higher monocyte percentage than the treated ones ($P < 0.01$). Regarding the serum lipids, the triglycerides tended to be more abundant in the F than in the M ($P = 0.061$), while cholesterol tended to be lower in the treated groups than in the C groups ($P = 0.091$). As for the serum minerals and the liver and renal enzymes, the live larvae supplementation only influenced the GGT (U/I), which was lower in the treated groups than in the C groups ($P < 0.05$) (Table 6). No significant effects were observed for the other blood parameters for either the fixed factors (gender and diet) or for the interaction between the gender and diet ($P < 0.05$).

# Discussion

## Growth performance

In the present study, the LW, ADG, and ADFI were higher, and the FCR was better in the M than in the F ($P < 0.01$). These results are in agreement with those reported in literature

TABLE 6 The serum minerals and the liver and renal functions of the male and female Label Rouge Naked Neck birds fed a diet supplemented with 10% live black soldier fly larvae; supplementation based on the expected daily feed intake (28–81d; $n = 12$).

| Item | Diet (D) | | SEM | Gender (G) | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | BSFL | Control | | Male | Female | | D | G | D x G |
| Liver function | | | | | | | | | |
| ALT, U/l | 9.95 | 11.7 | 1.03 | 11.08 | 10.6 | 1.03 | 0.220 | 0.755 | 0.383 |
| AST, U/l | 167 | 145 | 10.27 | 160 | 152 | 10.27 | 0.134 | 0.606 | 0.079 |
| GGT, U/l | 22.8 | 26.9 | 1.13 | 26.1 | 23.6 | 1.13 | 0.011 | 0.114 | 0.792 |
| Renal function | | | | | | | | | |
| Creatinine, mg/dL | 0.46 | 0.47 | 0.03 | 0.49 | 0.44 | 0.03 | 0.811 | 0.148 | 0.561 |
| Uric acid, mg/dL | 8.94 | 8.95 | 0.59 | 8.81 | 9.07 | 0.59 | 0.986 | 0.752 | 0.936 |
| Minerals | | | | | | | | | |
| P, mg/dL | 8.97 | 9.14 | 0.31 | 8.85 | 9.26 | 0.31 | 0.695 | 0.349 | 0.294 |
| Fe, µg/dL | 101 | 94.2 | 17.76 | 85.5 | 109 | 17.76 | 0.785 | 0.336 | 0.300 |
| Mg, mg/dL | 1.22 | 1.10 | 0.23 | 1.04 | 1.28 | 0.23 | 0.696 | 0.450 | 0.796 |

BSFL, black soldier fly larvae; SEM, standard error of the mean; ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; GGT, gamma-glutamyl transferase; CRE, creatinine; P, phosphorous; Fe, iron; Mg, magnesium.

for different growth rates in both chicken hybrids and chicken breeds (30–32). The LW did not differ among the groups at the beginning of the trial, but it was higher in the treated birds than in the controls at the end of the rearing cycle. Interestingly, such differences were already visible at 42 days of age, thus also pointing out advantages for birds slaughtered early. However, the larvae were not included in the formulation of the diet, with a consequent difference in the average nutrient intake among L and C groups (CP 24.24 g/d, EE 6.52 g/d, and GE 2.00 MJ/d vs. CP 23.02 g/d, EE 5.62 g/d, and GE 1.94 MJ/d on an as fed basis, respectively). Therefore, the difference in the LW could be explained by the difference in the CP content of the diets. A higher final LW was reported at 5 weeks of age by Veldkamp and van Niekerk (13) in a trial conducted on turkeys fed 10% live BSFL. However, the results obtained in chicken experiments are inconstant. For instance, Bellezza Oddon et al. (15) observed no effects on the growth performance of broilers fed 5% live BSFL. Dabbou et al. (33) instead reported a higher LW at 10, 24 and 35 days of age in broiler M fed 10% BSFL defatted meal, while de Souza Vilela et al. (34) found an increase in the body weight of broilers fed 20% BSFL full-fat meal. Since live larvae contain around 70% of water (35), the nutrients of live larvae are more diluted than in a larvae meal, which might make the effect of live larvae less visible over brief periods. The overall FCR of the LF was higher than the CF and the LM ($P < 0.05$ and $P < 0.001$, respectively), while the LM tended to show a better FCR than the CM ($P = 0.058$), thus suggesting a beneficial use of live BSFL, albeit only for the M. However, the real percentage of larvae ingested on the basis of the DFI was 10.41% for the F and 8.01% for the M, although the expected DFI between the M and the F was identical (19) and, consequently, so were the grams of live larvae administered to the birds. Moreover, the observed ADFI differed between sexes, and the M displayed higher values than the F ($P < 0.001$). Therefore, the FCR results could be related to the different percentages of larvae provided, resulting in lower % of BSFL needed to obtain benefits in this parameter. However, once again, the published data are inconstant, and are mainly focused on insect meal studies. De Souza Vilela et al. (34) reported a decrease of 10% in the FCR of broilers administered 20% BSFL meal. Moreover, Gasco et al. (36) have stated that levels of BSFL meal of up to 15% are not disadvantageous for the growth of birds. No differences were reported for the weekly measurements of the growth performance, except at the beginning of the trial, where greater performances were observed for the supplemented birds (ADFI at 28–35 d and 35–42 d; ADG and FCR at 35–42 d) and mostly for the M (ADG and FCR at 28–35 d), thus suggesting an advantageous provision of live BSFL, especially for young M (Supplementary Table S1).

## Larvae consumption duration

Live insects are a natural source of food for chickens and their administration in rearing systems may represent an environmental enrichment and a positive stimulus for poultry behavior (6). The attractiveness of larvae is influenced by their motility, which draws the attention of chickens (9). The larvae consumption duration had previously been evaluated in trials conducted by Veldkamp and van Niekerk (13) and Bellezza Oddon et al. (15) in turkeys and broilers, respectively, but the inclusion of the gender as a fixed factor has never been considered before. The social behavior of birds might be involved. The pecking order is mainly established during the first weeks of age, and the thus created dominance relationships tend to be maintained, especially in F (37). As a consequence, the larvae consumption dynamics could be more constant in F, which could eat larvae calmer than M. The larvae consumption duration of the birds was significantly higher for both the M and the F at the beginning of the trial than at the other considered periods. Since an unknown object was introduced into the chicken pen, some time for adaptation and neophobia resolution was expected, as was its reduction at the end of the experiment. However, the time dedicated to larvae consumption at the end of the experiment (7.19 and 2.11 min for F and M, respectively) was not as low as that registered for turkeys (below 2 min) by Veldkamp and van Niekerk (13). Additionally, the larvae consumption duration reported for broilers (around 6.5 min; (15) was lower than the F consumption time of this trial, but higher than that of the M. Such differences are probably related to social behavior (37). Moreover, genetic selection could also affect the behavioral pattern of chickens (38, 39), which could explain the differences from the larvae consumption duration of the other studies. However, further investigations are required to confirm this hypothesis.

## Blood traits

Overall, the live administration of BSFL did not negatively influence the blood traits of the birds, as has already been reported in other studies conducted with both BSFL meal and live larvae in different poultry species (13, 16, 30, 57). The GGT was detected in lower concentrations in the supplemented groups than in the controls ($P < 0.05$). Since the GGT concentration in plasma represents an indicator of the health status of the liver, this result suggests a beneficial effect of live BSFL on this organ (40). The leukocyte concentration, as well as the monocyte one, was higher in the groups fed BSFL than in the controls ($P < 0.05$ and $P < 0.01$, respectively). However, contradictory results were obtained in previous studies, which did not report any significant differences related to meal inclusion or live BSFL provision (15, 16, 33, 41). On the other hand, de Souza Vilela et al. (34) reported a reduction in the white blood cells of broilers fed increasing levels (0.5, 10, 15, and 20%) of full-fat BSFL meal, thus suggesting a positive effect on the immune system. However, no definitive conclusion can be drawn on this section and further research is needed. Finally, eosinophils (%) were higher in the F than in the M, whereas the opposite was found for monocytes (%). Differences between sexes in chicken blood values have already been found. Peters et al. (42) reported generally lower mean values of the hematological parameters (red blood cell count, hemoglobin, packed cell volume, white blood cell count, mean corpuscular volume, mean corpuscular hemoglobin concentration, serum glucose, urea, cholesterol, albumin, globulin and creatinine) in F than in M, while Addass et al. (43) reported higher levels of white blood cells in F than in M, although such differences might vary among chicken genotypes (18). However, the hematological parameters in the present study are in line with those reported in previous studies conducted on chickens and ducks fed live insects and insect meals (15, 16, 33, 44). The triglycerides tended to be more abundant in the F than in the M ($P = 0.061$), although Whitehead and Griffin (45) and Musa et al. (46) did not observe any differences in the blood triglyceride level between sexes in broilers and two Chinese chicken breeds. However, a high level of triglycerides in the blood could lead to an increase in the abdominal fat deposition (47), according to what has been found in the current research, as the abdominal fat of the F was double that of the M (2.02 vs. 1.01%, respectively). The cholesterol tended to be lower in the treated groups than in the C groups. Similar results were found for laying hens (48, 49) and Muscovy ducks fed insect meal (16). This result is probably related to the chitin content of the larvae, which could bind the cholesterol and significantly reduce its presence in blood (50, 51).

## Slaughtering performance

Overall, the gender had a significant effect on the carcass traits of the birds, with M showing better values than F for the SW, and the thigh yield (%CC weight). However, the F showed a higher breast yield (%CC weight) than the M. Such differences, which are related to sexual dimorphism, were also reported in previous studies conducted on both broilers and local Italian chicken breeds (52–55). The relative weight of the spleen (%SW) was improved as a result of the supplementation of live BSFL and was heavier in the treated groups than in the controls. Similarly, the relative weight of the Bursa of Fabricius (%SW) was higher in the LF than in the CF and the LM. The weight of these organs, which are involved in the immune system response, could be considered as an indicator of the immunity system activity. Moreover, stressed birds generally show a reduction in the dimensions of their organs due to a corticosterone effect (49, 56). Considering these results, the provision of live BSFL could be beneficial for the immune system, probably because of its positive effect on bird welfare (improving birds' immune system reaction to stressors), as well as the larval chitin content, which has an immunostimulant effect (49, 57). Similar findings have been reported by Bellezza Oddon et al. (15) for broiler chickens. However, the bursa of Fabricius was only affected by the diet in the female group, thus suggesting a higher sensitivity to the chitin effect in F than in M. Indeed, Glick (58) reported that a physiological reduction of the bursa of Fabricius dimension occurred during chicken growth, which was more marked in M, due to the development of testicles and the related testosterone activity. The relative weight of the organs (%SW) in our study varied between sexes. The gender was responsible for a statistical trend on the relative weight of the liver that tended to be higher in the F than in the M, in a similarly way to what was reported by Mosca et al. (53) for the liver weight (g) in a study conducted on the Milanino chicken breed reared under free-range conditions. Benyi et al. (52) instead reported a heavier liver (g) in males than in female broiler chickens, whereas Olawumi et al. (59) found no differences in broilers. Moreover, the F displayed a heavier relative weight of the heart (%SW) than the M, although previous research reported no differences between gender for this parameter (52). Finally, the heart tended to be heavier in the treated groups than in the controls. Since no similar findings have been reported in previous articles related to insect supplementation, and inconsistent results have been provided in other studies, a univocal hypothesis cannot be drawn. For instance, Badmus et al. (60) observed an increase in the relative weight of the heart (% body weight) in broilers supplemented with dietary corticosterone at 4 and 6 weeks of age. Lin et al. (61) instead reported a decrease in the heart weight of corticosterone-supplemented broiler chickens after 7 days of administration, but not after 11, when compared to the control groups. Thus, the heart weight increase can be either negative or positive, and this aspect requires further investigation. The abdominal fat was more abundant in the F than in the M, thus confirming the previous results of Shahin and Elazeem (62) and Benyi et al. (52).

## Conclusions

This study has revealed, for the first time, the potential of the provision of live BSFL on the performance and blood traits of medium-growing chickens. In the study, such a supplementation did not undermine the performance or the health of the birds. Additionally, the LW of the chickens benefitted from this supplementation, as did the FCR, ADG, and ADFI of the young birds, with more advantages observed in males than in females. Moreover, such insects, likely because of their chitin content, might have a positive effect on the hepatic function, as they reduced the GGT concentration in the blood. Finally, the immune system was ameliorated by the provision of live BSFL, increasing the bursa of Fabricius and spleen dimensions. However, further investigations are needed to clarify the effects related to the provision of live BSFL to medium and slow-growing chicken genotypes.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

## Ethics statement

The animal study was reviewed and approved by BioEthical Committee of the University of Turin (Italy) *Via* Verdi 8, 10124, Turin (Italy).

## Author contributions

FG, AS, VB, MG, and IB designed the experiment. VB, MG, EC, and VZ took care of the animal rearing. CC provided the live black soldier fly larvae. VB and IB performed the statistical analysis. VB wrote the first draft of the manuscript. AS supervised the study. All authors carried out the *post-mortem* analyses, contributed to the creation of the manuscript, and approved the submitted version.

## Funding

The authors would like to acknowledge the financial support received for this project from transnational funding bodies, partners of the H2020 ERA-NETs SUSFOOD2 and CORE Organic Cofund, under Joint SUSFOOD2/CORE Organic Call 2019, as part of the Poultrynsect project (Project No. 48).

## Acknowledgments

The authors are grateful to INAGRO (Belgium) technicians for the coordination of the rearing and shipment of the live larvae and to Mr. Dario Sola for the chicken management.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.1064017/full#supplementary-material

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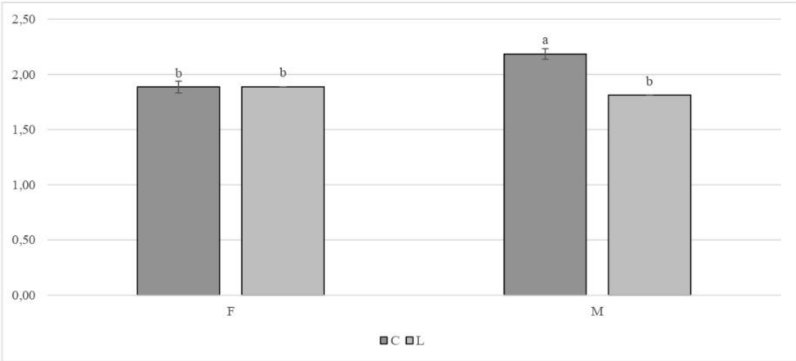
Supplementary materials

**Table S1.** Weekly growth performance of males and females Label naked neck birds fed a diet supplemented with 10% live black soldier fly larvae supplementation based on the expected daily feed intake, (28-81d; n = 6).

| Item | Days | Diet (D) | | Gender (G) | | SEM | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BSFL | Control | Male | Female | D | G | D×G | D | G | D×G |
| LW g | 28 | 479 | 475 | 515 | 439 | 2.07 | 2.07 | 2.92 | 0.186 | <0.001 | 0.298 |
| | 35 | 692 | 683 | 757 | 618 | 3.93 | 3.93 | 5.56 | 0.082 | <0.001 | 0.198 |
| | 42 | 939 | 924 | 1034 | 829 | 5.06 | 5.06 | 7.15 | 0.031 | <0.001 | 0.150 |
| | 49 | 1207 | 1185 | 1343 | 1049 | 7.03 | 7.03 | 9.93 | 0.022 | <0.001 | 0.527 |
| | 56 | 1498 | 1472 | 1684 | 1285 | 7.85 | 7.85 | 11.10 | 0.020 | <0.001 | 0.989 |
| | 63 | 1772 | 1744 | 2006 | 1510 | 10.55 | 10.55 | 14.93 | 0.062 | <0.001 | 0.759 |
| | 70 | 2040 | 2013 | 2333 | 1719 | 11.57 | 11.57 | 16.32 | 0.102 | <0.001 | 0.325 |
| | 77 | 2244 | 2212 | 2584 | 1872 | 11.36 | 11.36 | 16.05 | 0.050 | <0.001 | 0.868 |
| | 81 | 2372 | 2340 | 2742 | 1970 | 11.40 | 11.40 | 16.12 | 0.047 | <0.001 | 0.281 |
| ADG g/d | 28-35 | 31 | 30.1 | 34.6 | 26.4 | 0.36 | 0.36 | 0.51 | 0.070 | <0.001 | 0.008 |
| | 35-42 | 35.2 | 34.4 | 39.6 | 30.1 | 0.30 | 0.30 | 0.42 | 0.049 | <0.001 | 0.286 |
| | 42-49 | 38.2 | 37.2 | 44.1 | 31.4 | 0.41 | 0.41 | 0.57 | 0.071 | <0.001 | 0.320 |
| | 49-56 | 41.8 | 40.8 | 48.9 | 33.6 | 0.53 | 0.53 | 0.72 | 0.159 | <0.001 | 0.730 |
| | 56-63 | 39.1 | 38.8 | 45.9 | 32.1 | 0.67 | 0.67 | 0.95 | 0.761 | <0.001 | 0.476 |
| | 63-70 | 38.5 | 37.5 | 46.3 | 29.7 | 0.91 | 0.91 | 38.02 | 0.459 | <0.001 | 0.426 |
| | 70-77 | 29.5 | 30.2 | 36.3 | 23.5 | 0.91 | 0.91 | 1.29 | 0.606 | <0.001 | 0.853 |
| | 77-81 | 29.5 | 28.8 | 36.8 | 21.5 | 1.20 | 1.20 | 1.70 | 0.700 | <0.001 | 0.254 |
| ADFI g/d | 28-35 | 60.6 | 63.7 | 70.1 | 54.2 | 0.68 | 0.68 | 0.96 | 0.002 | <0.001 | 0.374 |
| | 35-42 | 78.1 | 80.6 | 89.4 | 69.2 | 0.71 | 0.71 | 1.00 | 0.010 | <0.001 | 0.605 |
| | 42-49 | 95.1 | 97.3 | 108 | 83.6 | 1.05 | 1.05 | 1.48 | 0.139 | <0.001 | 0.561 |
| | 49-56 | 105 | 108 | 122 | 91.5 | 0.99 | 0.99 | 1.40 | 0.069 | <0.001 | 0.068 |
| | 56-63 | 124 | 126 | 138 | 111 | 1.40 | 1.40 | 1.98 | 0.350 | <0.001 | 0.045 |
| | 63-70 | 135 | 133 | 152 | 116 | 1.44 | 1.44 | 2.03 | 0.493 | <0.001 | 0.210 |
| | 70-77 | 153 | 150 | 164 | 139 | 2.40 | 2.40 | 2.40 | 0.426 | <0.001 | 0.020 |
| | 77-81 | 144 | 146 | 163 | 126 | 3.32 | 3.32 | 4.69 | 0.654 | <0.001 | 0.175 |
| FCR g/g | 28-35 | 1.85 | 2.04 | 2.00 | 1.89 | 0.04 | 0.04 | 0.05 | <0.001 | 0.024 | <0.001 |
| | 35-42 | 2.09 | 2.14 | 2.08 | 2.15 | 0.02 | 0.02 | 0.03 | 0.042 | 0.015 | 0.643 |
| | 42-49 | 2.34 | 2.39 | 2.27 | 2.46 | 0.02 | 0.02 | 0.03 | 0.073 | <0.001 | 0.289 |
| | 49-56 | 2.38 | 2.41 | 2.28 | 2.51 | 0.02 | 0.02 | 0.03 | 0.474 | <0.001 | 0.926 |
| | 56-63 | 3.02 | 2.95 | 2.79 | 3.18 | 0.04 | 0.04 | 0.06 | 0.278 | <0.001 | 0.212 |
| | 63-70 | 3.26 | 3.41 | 3.06 | 3.61 | 0.06 | 0.06 | 0.08 | 0.063 | <0.001 | 0.002 |
| | 70-77 | 5.09 | 4.67 | 4.20 | 5.55 | 0.16 | 0.16 | 0.23 | 0.067 | <0.001 | 0.091 |
| | 77-81 | 4.81 | 4.89 | 4.12 | 5.58 | 0.2 | 0.2 | 0.3 | 0.769 | <0.001 | 0.472 |

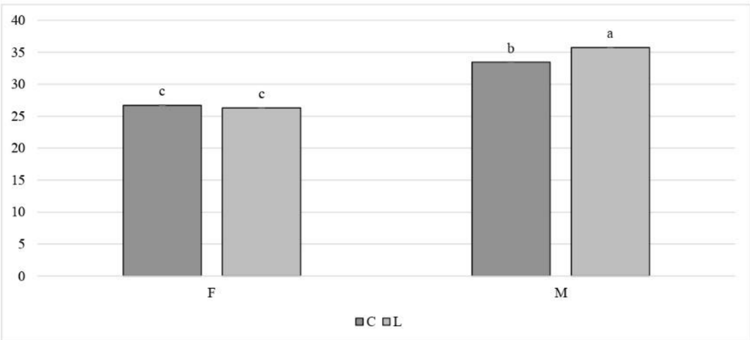
Abbreviations: BSFL, black soldier fly larvae; LW, live weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio (on a dry matter basis, including the larvae intake); SEM, standard error of the mean.

**Figure S1.** Interaction effect (gender×diet) on the feed conversion ratio at 28-35 days of age of the Label Rouge Naked Neck birds fed a diet supplemented with 10% live black soldier fly larvae; supplementation based on the expected daily feed intake, (28-81d; n = 6).




a,b indicates significant differences among groups, P<0.05. F: females; M: males; C: control; L: larvae.

**Figure S2.** Interaction effect (gender×diet) on the average daily gain at 28-35 days of age of the Label Rouge Naked Neck birds fed a diet supplemented with 10% live black soldier fly larvae; supplementation based on the expected daily feed intake, (28-81d; n = 6).




a,b,c indicates significant differences among groups, P<0.05. F: females; M: males; C: control; L: larvae.

## 10. Organic medium-growing chickens fed live black soldier fly larvae: towards fear reduction and enhanced activity and explorative behaviour

(submitted to Journal od animal Physiology and Animal Nutrition on July XX, 2023. Revision R1 submitted on October 10, 2023; current status revision R2; change after revision are in blue)

**Organic medium-growing chickens fed live black soldier fly larvae: a welfare improvement study**

**Running title: Live insects as environmental enrichment for poultry**

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## Summary

The overall beneficial effect of live black soldier fly larvae (BSFL) on the welfare of broiler chickens, turkeys, and laying hens has already been pointed out in the literature. However, scant information is available regarding medium-growing chicken hybrids reared under organic/free-range conditions, whose welfare has frequently been underrated. The aim of this research has been to provide additional information on this topic. Therefore, 240 label naked neck birds (Hubbard JA57 hybrid) were assigned, at 21 days of age, to four experimental groups (6 replicates/treatment, 10 chickens/replicate), created according to the sex and presence/absence of a 10% live BSFL dietary supplementation (control males, control females, larvae males, and larvae females), and raised until 82 days of age. Behavioural observations, a tonic immobility, and an avoidance distance test were performed. The feather damage and cleanliness, hock burn, footpad dermatitis, and skin lesion scores, as well as the excreta corticosterone metabolites (ECM) and heterophile/lymphocyte (H/L) ratio were determined. The behavioural observations demonstrated an overall increased physical and foraging activity ($P<0.05$) related to the live BSFL administration, representing valuable results on the explorative and recreational behaviour of this chicken genotypes. Moreover, the results pointed out the usefulness of

Abbreviations: BSFL, black soldier fly larvae; LNN, label naked neck; C, control groups; L, groups supplemented with live BSFL; CM, control males; CF, control females; LF, females supplemented with live BSFL; LM, males supplemented with live BSFL; CP, crude protein; AMEn: nitrogen-corrected apparent metabolizable energy; T1, time 1; T2, time 2; T3, time 3; T4, time 4; AD, avoidance distance; TI, tonic immobility; FPD, footpad dermatitis; HB, hock burn; ECM, excreta corticosterone metabolites; H/L, heterophile/lymphocyte; D×S, diet×sex; S×T, sex×time; D×T, diet×time.

live BSFL as a fear reducer in females as they moved closer to an operator during the avoidance distance test ($P<0.01$) after BSFL administration. No physical injuries or damage were observed on the birds, regardless of whether they were supplemented with live BSFL or not. The ECM were not affected by the live BSFL supplementation, while the H/L ratio was higher in the larvae groups than in the control ones ($P=0.050$). In conclusion, live BSFL could represent a mighty tool to improve life quality in medium-growing chickens, although follow-up research is required to clarify the stress modulation role of live BSFL on poultry production.

**Keywords:** environmental enrichment, poultry behaviour, organic farming, alternative rearing systems, *Hermetia illucens* larvae, free-range chickens.

**Highlights**

- Greater activity and foraging behaviour are observed in chickens fed live larvae.
- The use of live larvae could reduce the fear of medium-growing chickens.
- Live larvae provision does not interfere with the physical health of birds (plumage, hock burn, footpad dermatitis, and skin).
- Higher H/L ratio is observed in chickens fed live larvae.

## 1. Introduction

High productivity, the stocking density and environmental conditions, such as litter quality, are undeniably the predominant factors that generate welfare issues in poultry (EFSA, 2023). Although such matters have mainly been ascribed to fast-growing genotypes, birds reared in organic and free-range rearing systems are not exempt from these problems, especially when the breeding and management practices are not adequate (van de Weerd et al., 2009). Environmental enrichments represent a consolidated way of improving chicken welfare (Riber et al., 2018). Various approaches have already been tested as enrichment devices to favour the expression of the behavioural repertoire of birds and reduce the aggressiveness instances (Star et al., 2020), as well as their state of fear (Baxter et al., 2019). Inert materials, such as sand, rice hulls, wood and moss-peat shavings, can enrich the environment, albeit with some limitations, since they are devoid of a considerable feature of living beings: the capacity of motion (Riber et al., 2018). Live insects can fully satisfy the behavioural requirements of poultry in farming conditions through an environmental enrichment design (Gariglio et al., 2023; Schiavone and Castillo, 2023; Carr, 2016). The movement of insects can elicit the curiosity of chickens. Moreover, chickens spend a remarkable amount of time foraging and pecking the ground, and willingly eat insects, which are a natural part of their diet (Star et al., 2020). Concerning the insect species currently allowed for animal production purposes in the European Union, the BSF (*Hermetia illucens*) was chosen because of its features, spanning from environmental sustainability to animal welfare principles. These refer to the nutrient properties of BSF larvae (BSFL) (especially their protein and fat contents) and their bioconversion and food waste valorisation ability, as well as their satisfactorily employment in chickens' welfare improvement (Gasco et al., 2020; Purkayastha and Sarkar, 2021). Various studies have tested the effects of the provision of live larvae on poultry welfare. However, the poultry category encompasses several bird species, characterized by broad behavioural patterns and needs, which may elicit different responses. Veldkamp and van Niekerk (2019), for instance, conducted the first poultry study encompassing live larvae as environmental enrichment. They observed a reduction in feather pecking of the back and tail of turkeys supplemented with 10% of BSFL, when compared to the controls at five weeks of age. Similarly, Star et al. (2020) reported a better plumage condition of laying hens administered 10% live BSFL, in comparison to the control groups. Tahmtani et al. (2021) also investigated laying hens and found no change in behaviour of birds fed 10%, 20% and *ad libitum* live BSFL. Moving to broiler chickens, Ipema et al. (2020a) observed that birds fed 5% or 10% live BSFL exhibited a more intense foraging behaviour and were broadly more active than the controls. Biasato et al. (2022) demonstrated that a 5% dietary inclusion of live BSFL and yellow mealworm (*Tenebrio molitor*) larvae exerted beneficial effects on broiler chickens as it reduced the birds' fear and increased their foraging and activity behaviour, whereas no effects were observed on the ECM or on the H/L ratio during the experiment (Bellezza Oddon et al., 2021; Biasato et al., 2022). Nonetheless, no data are available about medium-growing genotypes reared in organic farming systems. It has been proved that medium- and slow-growing genotypes exploit higher activity levels in terms of ambulation, foraging, and overall exploration. This context resembles more the behavioural pattern of their ancestors compared to fast-growing genotypes, thus leaving a knowledge gap in the satisfaction of their behavioural and welfare requirements. In fact, the latter are often well identified but neglected, due to the wide and incorrect interpretation of the term "organic" as an animal welfare warranty (van de Weerd et al., 2009; Riber et al., 2018). Furthermore, this is the first study in which the evaluation of live larvae supplementation has been evaluated in both sexes simultaneously, which represents an important breakthrough in poultry research, being the females' breeding component a valuable part of the meat production chain not proportionally represented in welfare and behavioural broiler chickens' studies, especially in alternative rearing systems since the golden standard is usually the male. Broiler chicken females could display a different behavioural profile and reaction to the surrounding stimuli than males, due to the distinct attitudes characterizing the two sexes in their ancestors (e.g. males' boldness and females' prudence) (Collias and Collias, 1967). Therefore, this research has been aimed at investigating the behaviour and welfare implications of administering live BSFL-supplemented diets to organic medium-growing chicken hybrids, and an improvement in the activity and explorative behaviour as well as a reduction in stress and fear of the birds have been hypothesised.

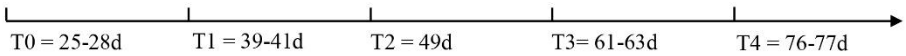
## 2. Materials and methods

### 2.1 Ethics

The animal study was reviewed and approved by the BioEthical Committee of the University of Turin, Via Verdi 8, 10124, Turin (Italy) (Prot. n°814715), in respect of the guidelines drawn up by Sherwin et al. (2003).

### 2.2 Animals and experimental design

The study was conducted at the poultry experimental facility of the University of Turin (Department of Agricultural, Forest and Food Sciences) (north-west Italy, 44.88572, 7.68381). A detailed description of the adopted experimental design and chicken management procedures was provided by Bongiorno et al. (2022); the same birds were considered for the previously mentioned experimental trial and the present one. Briefly, a total of 240 1-day-old LNN birds (Hubbard JA57 hybrid) were divided, according to their sex (M and F) and live BSFL supplementation in the diet (C or L) and reared from 21 to 82 days of age. Thus, there were four experimental groups (6 replicates/treatment, 10 birds/replicate), which were balanced for the live weight of the birds: CM, CF, LM, and LF. The birds belonging to the LM and LF groups received a 10% dietary supplementation of live BSFL, calculated on the basis of the daily estimated feed intake of the birds (Hubbard, 2021). Since the larvae were provided in addition to the feed, they were not considered in the diet formulation. A first-period diet was provided from 1 to 28 days of age (22.92/100 g of CP, 15.36 MJ/kg of AMEn as fed), and then a second-period diet was administered from 28 to 82 days of age (20.55/100 g CP, 14.19 MJ/kg of AMEn as fed) (Verzuolo Biomangimi S.r.l. – Verzuolo, CN, Italy). Each pen measured 2.2 m × 3.5 m, and rice husk was used as the bedding material. Furthermore, black cloths were used to cover the side walls of the pens for the whole trial duration to avoid the visual contact between birds housed in different pens. The birds in each pen had access to an external paddock (2.2 m × 4.5 m), from 49 days of age until the end of the trial, to ensure that the birds could spend one third of their lives in an outdoor area, according to the European Regulations on organic production (EU Regulation n. 889/2008). The insect larvae were administered to the birds daily (excluding Sundays) from 28 to 81 days of age at 11.00 a.m. The larvae were distributed on two plates (18.8 linear cm, 141.3 $cm^2$/chick, and 30 cm diameter). Two empty plates were introduced into the C bird pens to simulate the same interaction between the birds and the operator, thus maintaining the live BSFL provision as the solely difference between C and L groups (Bongiorno et al., 2022). The sampling days were clustered in order to attribute the same sampling time to each consecutive sampling day (T0 = 25-28 days of age; T1 = 39-41 days of age; T2 = 49 days of age, T3= 61-63 days of age; T4 = 76-77 days of age) to each bird.




**2.3 Behavioural observations**

Tablets were used to perform the video recordings at 25 (T0), 61 (T3), and 75 (T4) days of age. Four replicates/treatment were recorded and three time slots (5 minutes/each) were selected to check the birds' behaviour: in the morning (9.00 a.m.), during the provision of live BSFL (11.00 a.m.) and in the afternoon (4.00 p.m.), during the same day, and in the same registration order. The time samplings have been selected matching the ethogram identified as relevant for the present study and the technical resources available. Overall, the activity level of the birds is greater during the morning and late afternoon time than the rest of the day, both in domesticated chicken ancestors (Collias and Collias, 1967) and in commercial broiler strains (Weaver and Siegel, 1968; Foshee et al., 1970), despite further research is required to understand the birds' behavioural exploitation patterns through the day, based on the rearing environment and the resources provided (Bashir et al., 2023). In our case, we selected further times (compatible with the natural photoperiod to which the birds were subjected) both prior and after the live larvae provision. These two observations aimed at detecting indirect behavioural changes occurred in the supplemented groups, to validate the potential of live BSFL in stimulating natural behaviour even when no larvae were available anymore (e.g. prosecution of foraging activity to look for other larvae when all the ones provided were ingested). Moreover, to accomplish a direct evaluation of live BSFL influence on the birds, video recordings were carried out even during the live larvae administration. The video recordings were analysed by means of BORIS (Behavioural Observation Research Interactive Software v 7.9.7) (Friard and Gamba, 2016). The different kinds of behaviour were ordered into four macro groups: foraging related behaviour, comfort behaviour, activity behaviour, and social behaviour (Table 1). The occurrence of the specific types of behaviour was registered within each time slot (considering every continuous behavioural manifestation as one occurrence, regardless of its duration) and corrected by the number of birds observed in the pen every 30 seconds. The recorded ethogram was elaborated considering the previous studies conducted by Biasato et al. (2022), Ipema et al. (2020a), and Veldkamp and van Niekerk (2019).

<Insert Table 1>

### 2.4 Avoidance distance test

The AD test was performed, on the basis of the birds' response to the approach of humans, to measure their fear (Meuser et al., 2021). The same operator squatted on the litter close to a group of birds for 10 seconds and counted the number of chickens within a distance of 1m (arm's length) (Welfare Quality®, 2009), between 1m and 2m, and then over a distance of 2m from himself/herself. The test was executed at 27 (T0), 41 (T1), 62 (T3), and 76 (T4) days of age between 3.00 and 4.00 p.m.

### 2.5 Tonic immobility test

The TI test was performed to evaluate the level of fear of chickens, according to Dabbou et al. (2022). The test was always performed by the same operator in a separate area inside the building to avoid eye contact between the other birds. A total of three chickens/pen were randomly selected and labelled with a second wing mark at 26 days of age. These birds were then subjected to the TI test at 26 (T0), 39 (T1), 60 (T3), and 74 (T4) days of age. The test was performed according to the methodology adopted in a previous study (Campo et al., 2007). During the test, a bird was placed on its back on a U-shaped cradle. A slight pressure was applied to the breast of the bird and the time necessary for the bird to stop struggling and become immobile for at least for 10 seconds was recorded. If the bird righted itself in less than 10 seconds, the test was repeated for a maximum of 3 times. If TI was not induced within 3 attempts, the assigned score was 0 seconds. The maximum considered duration of TI was 10 minutes (600 seconds). Finally, the TI induction frequency was calculated on the basis of the number of inductions required to induce TI (from 1 to 3 attempts) and expressed as a percentage of the total of the executed attempts.

### 2.6 Feather damage and cleanliness

The plumage condition was always assessed by the same operator considering feather damage and breast feather cleanliness at 28 (T0), 49 (T2), 63 (T3), and 77 (T4) days of age. The former parameter was scored from 0 to 5, after evaluating the wings, tail, thighs and back covering conditions (Aviagen, 2014): 0 = fully feathered; 1 = rough; 2 = some broken feathers; 3 = heavily broken feathers; 4 = almost bald; 5 = bald. Feather cleanliness was instead scored from 0 to 4 (Welfare Quality®, 2009): 0 = clean; 1 = slight change in feather colouration; 2 = marked change in feather colouration; 3 = spotted litter and excreta stuck to the feathers; 4 = marked litter and excreta stuck to the feathers.

### 2.7 Leg health: hock burn, and footpad dermatitis scores

The leg health evaluation included both the FPD and the HB scores. The sampling times were the same as those adopted for the feather condition evaluation and skin lesions score, and every observation was carried out by the same operator. The FPD was scored as follows (Welfare Quality®, 2009): 0 = no lesions; 1 = minor and superficial lesions of the skin with hyperkeratosis; 2 = moderate and superficial lesions of the skin with hyperkeratosis (less than one quarter of the foot pad affected); 3 = severe and deep lesions with hyperkeratosis (one half of the foot pad altered); 4 = severe and deep lesions with hyperkeratosis (more than one half of the foot pad altered). The HB was instead evaluated as follows (Welfare Quality®, 2009): 0 = no lesions or mild skin rash; 1 = pronounced skin rash; 2 =

moderate skin lesions and blood scabs; 3 = severe but confined skin lesions and necrotic areas (less than one half of the area altered); 4 = severe and extended skin lesions and necrosis (one half or more than one half of the area affected).

### 2.8 Skin neck and breast lesion scores

The skin lesion condition was assessed concurrently with the feather condition. Two areas were considered regarding the skin lesion scoring: the neck and the breast. The scoring system adopted for the neck-skin lesions was designed as follows (Welfare Quality®, 2009): 0 = no lesions or fewer than three pecks (punctiform damage of less than 0.5 cm diameter) or scratches; 1 = at least one lesion of less than 2 cm diameter at the largest extent or three or more pecks or scratches; 2 = at least one lesion of 2 cm or more than 2 cm diameter at the greatest extension. The breast-skin lesion protocol was instead developed considering the presence or absence of erythema: 0 = normal skin colouration; 1= intense but contained breast-skin redness (less than one half); 2 = intense and extended breast-skin redness (one half or more than one half).

### 2.9 Excreta corticosterone metabolite analysis

Fresh excreta samples were collected individually at 26 (T0), 39 (T1) and 74 (T4) days of age considering two random birds selected for the TI test. Each bird was kept in a wire-mesh cage (100 cm width × 50 cm length) until at least 2 grams of fresh excreta had been produced and collected in a plastic box under the cage. Subsequently, the samples were stored at −20°C for the corticosterone analysis. The ECM was executed according to Costa et al. (2016) and Palme et al. (2013). Briefly, 3 mL of 80% methanol (Sigma Aldrich, St. Louis, MO, USA) was added to 0.25 g of lyophilized excreta in an extraction tube and maintained at -20°C for 2 h to allow the solid phase to settle on the bottom. After 2 h, the supernatant was transferred to another vial and evaporated under a hood for 14 h. The ECM was determined by means of a multi species enzyme immunoassay kit (K014 - Arbor Assay®, Ann Arbor, MI, United States) validated for serum, plasma, saliva, urine, dried faecal extracts, and tissue culture media. The inter- and intra-assay coefficients of variation did not exceed 10% and the sensitivity of the assay was 11.2 ng/g of excreta. Multiple dilutions were conducted to perform the sample analyses (1:4, 1:8, 1:16, and 1:32) and all the regression slopes were parallel to the standard curve ($R^2$ = 0.989). The mean recovery rate of corticosterone added to dried excreta was 96.5%.

According to the manufacturer, the corticosterone kit presents the following cross reactivity: 100% with corticosterone, 12.3% with desoxycorticosterone, 0.62% with aldosterone, 0.38% with cortisol and 0.24% with progesterone. All the analyses were performed in duplicate, and the concentration of ECM was expressed as ng/g excreta dry matter.

**2.10 H/L ratio**

At slaughtering time, during bleeding, blood samples were drawn from each of the 48 birds selected for the slaughtering at 82 days of age. An aliquot of 2.5 mL of blood was stored in a tube containing EDTA. Subsequently, a drop of blood was placed onto a glass slide and a smear was obtained. May-Grünwald and Giemsa stains (Campbell, 1995) were used to stain the smears, and a 1:200 Natt-Herrick solution was used to treat the samples (Natt and Herrick, 1952). The erythrocyte and leukocyte counts were defined using an improved Neubauer haemocytometer (Salamano et al., 2010). One hundred leukocytes, both granular (heterophil, eosinophil and basophil) and non-granular (lymphocyte and monocyte), were counted on the glass slide, and the H/L ratio was calculated.

**2.11 Statistical analysis**

The data analysis was performed using IBM SPSS Statistics (IBM Corp., 2011). Primarily, the outlier evaluation has been performed for each parameter considered, but no anomalous data were relieved and removed from the dataset. Subsequently, the data were tested for the homogeneity of variances by means of Levene's test, and both the residuals and data were tested for normality using Shapiro-Wilk's test. A total of 4 experimental groups, based on sex and live BSFL dietary supplementation, were considered. The experimental unit was the pen for each of the following parameters: feather condition, leg health, skin lesion scores, AD test, TI test, ECM (n=6 per treatment) and behavioural observations (n=4 per treatment). The same observer took care of the whole behavioural analyses of the birds, while another rater provided a second feedback on sequences of video samples during the video analyses process to assure the observation consistency. The observer was firstly taught about the importance of the scientific detachment and straight description of behaviours to look at within the observation protocol. Subsequently, the observer watched video recordings of replicates not involved in the statistical analyses as training. In addition, the video recordings were watched twice and no discrepancies in the obtained results were observed (intra-rater reliability ICC: ≥0.90) (Bateson and

Martin, 2021). Finally, the accurateness of the ethogram provided, and the simplicity of the activities monitored importantly contained the error margin may present within observations and raters (Anderson et al., 2014). The bird was instead considered as the experimental unit for the H/L ratio analyses and the correlation between TI duration and ECM content (n=12). Moreover, a general linear mixed model (GLMM) was fitted to analyse the feather condition, leg health, and the skin lesion scores (negative binomial response probability distribution with a nonlinear link function -log), TI duration, TI induction frequency and ECM (gamma probability distribution and log-link function), AD test and video recordings (Poisson loglinear distribution). The D, S, T, and their D×S, S×T and D×T interactions were considered as fixed factors (assessed by means of pairwise comparisons), and the replicate was included in the model as an indicator of the repeated measurements on the same pen. The frequency of the exploited behaviour is here intended as the number of behavioural observations performed during the recordings. A general linear model (GLM) was used to analyse the H/L ratio, considering S, D, and their interaction (D×S) as fixed factors. Finally, a Spearman correlation test was performed for the ECM concentration and the TI duration, being these data not normally distributed. All the data were reported as least square means and as the standard error of mean (SEM). Significant differences were declared for P values ≤0.05. Any behaviour and animal-based welfare measurements that were observed at a lower frequency than 0.5 times on average per period of observation were not subjected to statistical analyses and are thus not reported in the results.

## 3. Results

Overall, by virtue of the two plates provided, all the LM and LF birds had access to the larvae during their administration, thus ate the same amount of BSFL.

### 3.1 Behavioural observations

The behavioural observations made in the morning, during live BSFL provision, and in the afternoon are presented in Tables 2 and 3, and in Figures 2, 3, and 4

<Insert Table 2 and Table 3>

#### *3.1.1 Foraging-related behaviour*

In the morning, the foraging-related behaviour was influenced by the D×S and D×T interactions. CM showed more ground pecking behaviour than LM (6.04 *vs* 4.20 (P<0.001)) (F1Am). Furthermore, fewer L birds performed ground pecking than C birds at T0 (5.25 *vs* 10.81, P<0.001), while no differences were observed between the L and C birds at T3 and T4 (F1Bm). By contrast, the live BSFL administration significantly decreased the scratching behaviour in all groups (P<0.001) (Table 2).

In the afternoon, the ground pecking resulted to be more frequent at T3 and T4 than at T0, albeit only in the supplemented birds (P<0.05), with higher frequencies being observed in the L birds than in the C ones (T3: 11.25 *vs* 4.48, P=0.001; T4: 10.31 *vs* 7.47, P<0.001) (F1Al). The pecking object frequency in the L groups was instead lower than that of the C ones, albeit only at T0 (P<0.001) (F1Bl) and was greater in LM than in LF (P<0.05) (F1Cl). Similarly, the scratching frequency was also affected by the interaction D×S, being higher in LF than in LM (P<0.05) (F1Dl). Moreover, the scratching frequency was greater in the C groups than in the L ones at T0 (2.73 *vs* 0.79, P<0.05), while the opposite trend was observed at T3 (0.27 *vs* 1.46, P<0.05) (F1El).

Overall, the foraging-related behaviour in the afternoon observations was influenced by the diet and by the D×S, and D×T interactions. The CF birds showed a higher ground pecking frequency than the CM ones (6.19 *vs* 4.38, P=0.001), whereas no differences were noted between males and females when the birds were supplemented with live BSFL larvae (F1Aa). The scratching behaviour was also higher in the L groups than in the C groups, even though this was observed at T3 only (P<0.01) (F1Ba). Finally, the larva consumption frequency decreased between T3 and T4 (P=0.05) (Table 2).

<Insert Figure 1>

*3.1.2 Comfort behaviour*

The comfort behaviour frequencies were <0.5 (in the morning, during live BSFL provision and in the afternoon observations). Therefore, such behaviour was not analysed.

*3.1.3 Activity behaviour*

In the morning, the diet affected the walking behaviour of the female birds, with a greater number of LF birds walking than the CF ones (P=0.010) (F2Am). However, the walking and the standing frequencies abruptly decreased between T0 and T3, independently of the diet (P<0.001) (Table 2), while the L birds displayed a greater standing behaviour than the C ones (P<0.05) (Table 2). The resting frequency was significantly lower in the females than in the males (P<0.01), regardless of the experimental times (both in the morning and during larvae provision) (Table 2). More CM than CF birds were observed in the external paddock (1.62 *vs* 0.31, P<0.001), with no recorded differences between LM and LF (F2Bm). On the other hand, to conclude the morning observations, a greater number of LF than CF availed the outside space during the morning observations (0.99 *vs* 0.31, P<0.01) (F2Bm).

During the live BSFL provision, the standing frequency was higher in the LM groups than in the LF ones (22.78 *vs* 11.32, P<0.05) (F2Al). Furthermore, the standing frequency was observed to be higher at T0 and T3 than at T4 (P<0.01) (Table 2). The resting frequency increased instead as the birds became older (P<0.001) and was greater in males than females (P<0.001) (Table 2). Finally, during the larvae administration, more C birds explored the external paddock than L ones, albeit only at T4 (P<0.01) (F2Bl).

In the afternoon, the chickens' walking frequency decreased as they became older in all the groups (P<0.001) (Table 2). Moreover, the walking frequency was lower in the CM chickens than in the LM ones (13.66 *vs* 22.20, P<0.05) (F2Ba). Similarly, the standing frequency decreased with age in both C groups (for every sampling time) and L groups (between T3 and T4 only) (P<0.05), and it was higher in L birds than C ones at T4, while the opposite was true at T0 (P<0.001) (F2Aa). As far as external paddock utilisation is concerned, more females than males were observed outside in the C groups (5.52 *vs* 0.62, P<0.01), whereas the opposite was recorded for the L ones (0.58 *vs* 2.38, P<0.05) (F2Ca).

<Insert Figure 2>

*3.1.4 Social behaviour*

The social behaviour frequencies were <0.5 (in the morning, during live BSFL provision and in the afternoon observations). Therefore, such behaviour was not analysed.

**3.2 Ethological tests and animal-based welfare measurements**

*3.2.1 Feather condition, leg health, skin lesion scores*

Since damage to the birds' feathers, legs, feet, and skin occurred at a lower frequency than 0.5 times on average, these aspects cannot be considered for statistical analyses.

*3.2.2 Avoidance distance test*

The results of the AD test are reported in Table 3 and Figure 3. Since no chickens were observed within a distance of 1m from the operator, these data were not subjected to statistical analysis. Moreover, the data over 2m are not shown, since they were complementary and opposite the data pertaining to the birds observed within 1-2m. A greater number of males than females were always noted within 1-2m from the operator (4.83 *vs* 2.48, P<0.001), except at T3 when no differences between males and females were recorded (F3A). Moreover, the live BSFL supplementation increased the number of females within a distance of 1-2m from the operator (1.74 *vs* 3.52 for CF and LF, respectively) (P<0.01) (F3B).

*3.2.3 Tonic immobility test*

At T0, a lower percentage of L birds remained immobilised at the first TI attempt than the C ones (100 *vs* 94.27) (P<0.05) (F3C), while the TI duration increased between T1 and T3 for both males and females and in the C and L groups (P<0.001) (Table 3). Only at T0 did the L birds show a longer TI duration than the C ones (1.58 *vs* 1.16 min) (P<0.01) (F3D).

<insert figure 3>

*3.2.4 Excreta corticosterone metabolites*

Both sex and time had significant effects on the ECM content, which was lower in the females than in the males (36.6 *vs* 40.1 ng/g) and decreased over time (P<0.001) (Table 3). No significant correlations were found between the TI duration and the ECM content (data not shown).

*3.2.5 Heterophile/Lymphocyte ratio*

The H/L ratio was lower in the C groups than in the L ones (0.91 *vs* 1.27) (P=0.050), whereas no significant differences were detected for the other fixed and interaction factors (Table 3).

**4. Discussion**

Bongiorno et al. (2022) reported how live BSFL supplementation ameliorated the growth performance of medium-growing chickens (especially male birds), with benefits on the hepatic function and immune system. The present research has been aimed instead at highlighting the effects of the administration of live insects on the behaviour and welfare of medium-growing chickens.

**4.1 Behavioural observations**

*4.1.1 Foraging behaviour*

The provision of live BSFL decreased ground pecking in the male groups and the scratching frequency of all birds (morning), despite the opposite has been reported by Biasato et al. (2022), Ipema et al. (2020b), and Ipema et al. (2022) for broiler chickens, and by Veldkamp and van Niekerk (2019) for turkeys. However, the birds might reduce their foraging activity as an anticipation of the subsequent live BSFL provision, fully capable to satisfy their behavioural needs.

Interestingly, the ground pecking frequency was enhanced by the live BSFL during the administration period, thus suggesting an increase in the exploratory behaviour over a short-term period, and therefore a more advantageous practice if performed several times a day rather than once (Ipema et al., 2020a). The pecking and scratching frequencies increased in a similar way when comparing LF with CF. Unlike the in-field observations executed by Collias and Collias (1967) on Red Jungle Fowl, our investigation has also considered inspection of the surrounding food-sources by the dominant male, which was followed by a more frequent ground pecking and scratching of the hens in the cockerel's wake to pursue the search of food. No significant differences in exploratory activity were detected by Veldkamp and van Niekerk (2019), although several studies (Biasato et al., 2022; Ipema et al., 2020a; Ipema et al., 2020b) have reported that the provision of live larvae stimulates positive foraging behaviour. Our findings, which support the latter hypothesis, have shown that a greater number of L birds were observed scratching at T2, although the opposite trend was recorded at T0, when no larvae had as yet been provided. Similarly, a greater scratching frequency was observed in the L group than in the C groups at T3 (afternoon). Those findings underline how this enrichment can stimulate the natural behaviour of the birds, albeit starting from opposite trends or absence of differences before the live BSFL provision onset. Furthermore, a greater ground pecking frequency was noted in the CF groups than in the CM groups during the same period of the day, while no differences were detected between the supplemented groups of either sex. This result might be reconducted to the exploration stimulation of the birds generated by the presence of live BSFL, although the high SEM in the LM groups could be responsible for the insignificant differences observed between such groups and the LF ones. Finally, the reduction in the larva consumption frequency between T3 and T4 could be attributed to the lower activity of birds over time as well as to changes in the weather, since the trial was conducted in autumn-winter and the motility of larvae decreases at low temperatures, thus making them less attractive. In fact, during the trial, the average environmental temperature was 12.8°C (min 5°C; max 22°C) and 7.6°C (min −1°C; max 16°C) °C, in October and November 2021, respectively. The average relative humidity was 73.5% (min 52%; max 95%) and 70.5% (min 41%; max 100%) in October and November 2021, respectively], Moreover, a repeated, portioned, and consequently reduced amount of larvae distributed per each administration, may positively increase the larvae consumption frequency, and should be considered in future studies.

*4.1.2 Activity behaviour*

A higher walking frequency was noted in the LF groups than in the CF ones (morning), and in the LM groups than in the CM ones (afternoon), as a result of the beneficial effect of live BSFL provision on the birds' activity, as previously observed by other authors (Biasato et al., 2022, Ipema et al., 2020b, Ipema et al., 2022). Furthermore, a greater number of L birds displayed longer standing behaviour than the C ones (morning), while more LM birds were observed in a standing position than LF (during the larva administration). Since male birds reach heavier weights than females and tend to behave as regular broilers as their weight increases, thus reducing their activity level, the administration of live BSFL could be a promising tool to maintain the activity of these birds. In spite of these satisfactory results, the walking frequency overall decreased as the chickens became older (morning, afternoon) as did the standing frequency (morning, during live BSFL provision, afternoon), probably due to the weight the birds had gained (Bokkers and Koene, 2003; Jacobs et al., 2021). The resting frequency increased as the age of the birds increased during the larva administration in both males and females, which might be contradictory, as a greater activity of the birds could have been expected due to the provision of insects. However, the frequency indicates the number of times in which a certain type of behaviour is exploited. Therefore, the increased resting frequency followed a previous state of active behaviour, probably linked to the consumption of live BSFL, thus turning such behaviour into an indicator of the birds' activity. However, in the present study, the behavioural frequencies were considered but no time budget information (due to technical limitations related to the low camera resolution) was available to attribute the amount of time spent for each activity. Curiously, more LF than CF were observed outside (morning and afternoon), thus suggesting an enhancement in the exploring activity of the females related to the live BSFL supplementation, while more C birds than L ones availed the outside paddock (during the live BSFL provision) at T4, which might represent an anticipatory behaviour related to larva administration. To conclude this chapter, the challenge in interpretating the activity levels of the birds must be faced. Since we considered the frequency of the activities performed by the birds, we could not directly compare it to the behavioural budget available in literature. However, meaningful considerations can be drawn analysing the gathered data. In more detail, the number of observations recorded varied one from each other of about one third when statistically significant, if not doubling or tripling among the different times and diets, provide evidence of the biological relevance of our findings, held in consideration the reared genotype, the number of birds available and their yet good basal welfare conditions, the duration of the observations, and total number of the observation which always ranged within was always into the ten events per observations.

*4.1.3 Social behaviour*

The diet did not affect the social behaviour of the birds. The absence of negative interferences with the chickens' social patterns should be recognized as a positive result, since the live BSFL provision did not increase aggressive or competitive behaviour for their access. However, due to the high standard deviation, some results obtained may not be significant. This situation could derive from the behavioural variation identified between and within the groups, ascribable to the various chickens' personality (Taylor et al., 2017; Rufener et al., 2018; Sibanda et al., 2020).

**4.2 Feather condition, leg health, and skin lesion scores**

Since all birds had a basic good welfare status, no welfare issues related to the feather condition, leg health, or skin lesions occurred and all the scores were close to zero. However, a study limitation is identifiable in the low rearing density, thus reducing the visibility of the potential beneficial effect connected with the live BSFL supplementation. It is namely broadly recognized that the optimal basal behaviour conditions, e.g., low stocking density diminished lameness (Dawkins et al., 2004) along with improved leg health (Hall, 2001) and feather condition (van Hierden, 2003).

**4.3 Avoidance distance test**

In this research, we needed to adapt the Welfare Quality® protocol to our structures and our hybrid, which is more reactive than the fast-growing broilers. Due to the higher area/bird available when compared to a commercial farm, it was expectable that few/no birds would be observed within 1m. However, we noticed a clear distinction between the space occupied by the birds, since some of them were more prone to explore the "novelty" within the pen: the squatted operator. This fact created the need to insert the 1-2m and over 2m classes. The within 1-2m location itself is not necessarily positive or negative, but it assumes a positive meaning in this research since the birds had to face the human presence daily, both for management as well as during the live BSFL administration. Several levels of fear can be observed in poultry, depending on their breed, environmental conditions, and health, although a basic avoidance and phobia degree have been recognised as positive welfare indicators that have been inherited from Jungle fowl as a defence against attacks from predators (Linares and Martin, 2010). Therefore, the absence of chickens within 1 m from an operator can be intended as healthy bird behaviour (Muir et al., 2008).

Interesting results were observed for the 1-2m AD test, where a higher confidence of male birds (T0, T1, T4) than female ones was revealed, probably linked to an ancestral major audacity of males in exploring the surrounding environment, compared to females. This hypothesis, derived from the in-field study conducted by Collias and Collias (1967), states that the exploring-scheme of a flock is determined by the dominant cockerel walking in the position of leader through a jungle followed by the hens. Moreover, hens, being responsible for brooding and offspring protection, could be more prudent than M. This might explain why we noted less confidence towards the operator in the females than males, despite the same bird management practices. Nevertheless, the supplementation advantages were underlined by the gain in the human-animal relationship, as the LF birds moved closer to the operator than the CF ones did, thus counterbalancing the fear and the exploration behaviour, a fundamental objective of welfare-respectful rearing systems (Meuser et al., 2021). The present finding has beneficial implications within the "on-farm" reality, where the operator must interact with the animals daily, event that might be experienced as a stressor by the birds. However, the positive association of humans to a reward, as the larvae provision performed directly by the operator or even the visual presence of the operator when an automatic distribution occurs, could be beneficial in the regular daily management of the birds, with positive outcomes not only on chickens' welfare but even on their production. Our hypothesis is that once the females recognize humans as a source of feed, they might be more motivated in approach them due to their natural attitude in identify and select feed sources for the future offspring during the parental feeding (Stokes and Williams, 1971).

### 4.4 Tonic immobility test

The TI test is widely recognised as an indicator of the fear of birds (Ipema et al., 2020b). The administration of live BSFL had no effect on the tonic immobility duration, while an increase in the TI duration was observed during the considered experimental times in both sexes. Similar observations were made on broiler chicken breeders (Brake et al., 1994) and White Leghorns (Campo and Carnicer, 1993). Brake et al. (1994) assumed that the higher weight and the reduced activity level of adult birds were responsible for the prolonged TI over time, a hypothesis that matches perfectly with our findings.

### 4.5 Excreta corticosterone metabolites

ECM analysis is an accountable method that can be used to evaluate stress in birds (Touma and Palme, 2005) as it does not require restraining the birds or collecting blood (Weimer et al, 2018). In our study, the live BSFL did not result in variations in the ECM, although it was lower in the females than in the males, which might be related to a greater vigilant attitude of males than females. Similarly, Hirschenhauser et al. (2012) and Touma and Palme (2005) indicated that sex is a relevant factor that affects the corticosterone metabolites in droppings, with differences in the excretion peaks and compositions. Moreover, the ECM subsided with ageing, probably due to a developed habit of birds towards human contact, although further research on this topic is needed.

### 4.6 Heterophile/Lymphocyte ratio

The H/L ratio is a consolidated indicator of stress in poultry (Gross and Siegel, 1983; Mahboub et al., 2004). Surprisingly, the H/L ratio of the birds in the current work was lower in the C groups than in the L ones (P=0.050). However, considering the absence of exposure of the chickens to intense and prolonged stress conditions, the results of our research might not be related directed to a negative bird experience. On the other hand, a negative effect related to the competition for the access to larvae cannot be ruled out despite no aggressive or competing interactions have been observed during the video recordings. Another explanation could lay in the anticipatory behaviour of birds, being the stress positively attributable to the live larvae provision. Overall, this result sheds light on uncharted aspects about live BSFL in poultry production. Indeed, this appealing environmental enrichment and its utilization should be contextualized on the basis of the structural and managerial peculiarities which characterize each barn, and that are capable to both positively and negatively affect welfare and health of birds. In more detail, if and at which rate larvae can affect competition and anticipatory behaviour should be investigated, together with the influence of the administrations/day. Moreover, fruitful findings could derive from the distribution method adopted (larvae dispenser/ scattered larvae), with a special focus on the more active chickens' genotype. Finally, a clarification of the BSFL effect on birds' stress might be provided in future studies by the combination of this parameter with other interesting stress measurements, like the use of thermal imaging cameras (Nicole, 2020; Jacobs et al., 2023).

**5. Conclusions**

The number of reared birds in the study was determined by the production capacity of the larvae pilot plant. However, practical implications and interesting assumptions can be addressed on a commercial scale. For a start, the reduced density of the birds could have been, on one hand, responsible for the absence of significant results on plumage integrity and leg health, due to the overall good basal welfare conditions of the chickens. On the other hand, the presence of significant results for other several considered parameters, represents firm evidence of the beneficial impact of live larvae in poultry production. Furthermore, such effects can be amplified in rearing conditions where high welfare standards are more challenging to be maintained. This concept can be applied to the behavioural observations as well as the ethological tests. A valid instance is represented by the AD test. In fact, testing the birds in a pen where they have enough space to decide if and how closely approach the operator, provides a pure information about fear and exploration readiness of the birds. The data collected could hence be useful to determine the larvae effectiveness on farm, where the test itself might be biased by the greater density (the impossibility of birds to range in the barn as they would like to). In conclusion, this research presents novel information regarding the welfare and behaviour of medium-growing chickens fed live BSFL and farmed in a free-range organic system. It must be stated that the hybrid type and rearing system combination is commonly considered as a direct positive indicator of birds' welfare status. However, this perspective does not reflect a certainty in terms of animal welfare and exploitation of the chickens' behavioural repertoire. Therefore, improvement actions in medium-growing chickens' life conditions should be pursued. At this regard, positive effects were identified in this study on the fear regulation of birds by means of the AD test, and an enhancement in the chickens' exploration (mostly in the F) and locomotion activity was also observed. The obtained results find application on farm reality, confirming the live larvae potential in stimulating and satisfying the birds' behavioural necessities and keeping a healthy flock. Moreover, live BSFL could concretely reinforce the human-animal relationship, extremely useful to avoid stressful interactions among the operators and the birds, and promote instead chickens' performance.

## 6. Data availability statement

The original contributions presented in this research are included in both the article and Supplementary Materials. Further inquiries can be directed to the corresponding author.

## 7. Authors' contributions

FG, AS, VB, MG, and IB designed the experiment. VB, MG, EC, and VZ were in charge of the animal rearing and performed the tests/video recordings. EM and IM carried out the corticosterone analyses. SB performed the blood analyses. VB and IB performed the statistical analysis. VB and IB wrote the first draft of the manuscript. AS, MR, IB, and LG reviewed the manuscript. AS supervised the study. All the authors contributed to the creation of the manuscript and approved the submitted version.

## 8. Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.


## 9. Acknowledgements

The authors are grateful to the INAGRO (Belgium) technicians for the coordination of the rearing and shipment of the live larvae, to Mr. Dario Sola for the chicken management, and to the professors Maja Makagon and Joy A. Mench for the support in the elaboration of the data. This work was supported by transnational funding bodies, partners of the H2020 ERA-NETs SUSFOOD2 and CORE Organic

Cofund, under Joint SUSFOOD2/CORE Organic Call 2019, as part of the Poultrynsect project (Project No. 48).

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Table 1. Ethogram of the specific behaviour and activity of the studied chickens.

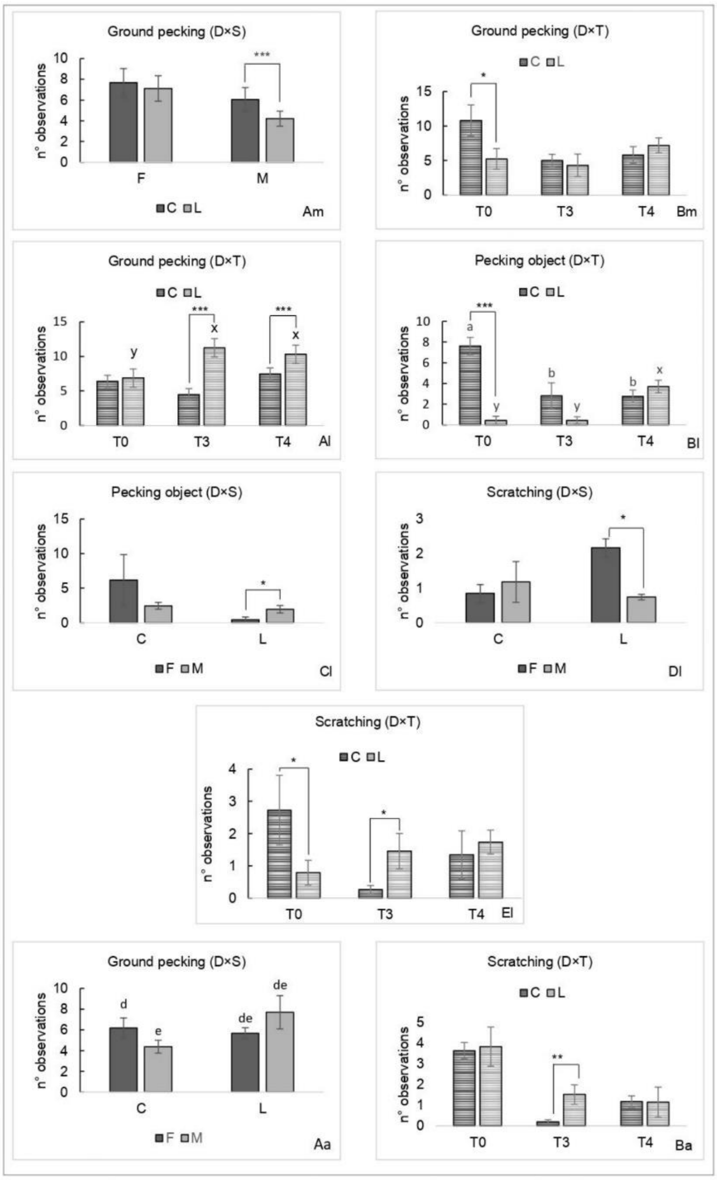
| Categories | Sub-categories | Description | References |
|---|---|---|---|
| Foraging related behaviour | Ground pecking | Pecking the ground | (Ipema et al., 2020a) |
| | Pecking object | Pecking objects | (Veldkamp and van Niekerk, 2019) |
| | Scratching | Moving litter backwards by means of the claws | (Biasato et al., 2022) |
| | Eating larvae | Pecking larvae from the plates | - |
| Comfort behaviour | Preening | Self-feather grooming by means of the beak | (McCowan et al., 2006) |
| Activity behaviour | Walking | Walking/running | (Biasato et al., 2022) |
| | Standing | Standing stationary | (Veldkamp and van Niekerk, 2019) |
| | Resting | Sitting/lying stationary | (Veldkamp and van Niekerk, 2019) |
| | Outside | Having access to the outside paddock | - |
| Social behaviour | Sparring/fighting | Play fighting/fighting | (Veldkamp and van Niekerk, 2019) |
| | Chasing | Running after a conspecific | (Biasato et al., 2022) |
| | Pecking conspecifics | Pecking movements directed at a pen mate | (McCowan et al., 2006) |
| | Allopreening | Social preening | (Kenny et al., 2017) |

Table 2. Effects of the live black soldier fly provision on the behaviour performed by both males (M) and females (F) of the medium growing hybrid label naked neck chicken, in relation to diet (D), sex (S), time (T), and their interactions, in the morning (m), during BSFL provision (l), and in the afternoon (a) (n=4).

| Class | Behaviour (n° birds) | Diet (D) | | Sex (S) | | Time (T) | | | SEM | | | P-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | L | F | M | T0 | T3 | T4 | D | S | T | D | S | T | D×S | D×T | S×T |
| During the morning | | | | | | | | | | | | | | | | | |
| FRB | Ground p. | 6.80 | 5.47 | 7.39 | 5.04 | 7.53 | 4.65 | 6.48 | 0.79 | 1.09 | 1.32 | <0.001 | 0.124 | 0.363 | 0.004 | 0.005 | <0.001 |
| | Object p. | 1.73 | 1.91 | 1.83 | 1.80 | 2.43 | 1.30 | 1.89 | 0.71 | 0.71 | 0.71 | 0.816 | 0.923 | 0.075 | 0.010 | 0.462 | 0.234 |
| | Scratching | 2.57 | 1.24 | 3.48 | 0.91 | 1.53 | 1.30 | 2.87 | 0.61 | 0.71 | 0.88 | 0.013 | <0.001 | 0.100 | 0.148 | 0.123 | <0.001 |
| CB | Preening | 4.35 | 4.43 | 4.90 | 3.94 | 3.38 | 4.86 | 5.16 | 0.64 | 0.58 | 0.90 | 0.926 | 0.096 | 0.114 | 0.227 | 0.104 | 0.651 |
| AB | Walking | 14.1 | 20.4 | 16.7 | 17.2 | $29.3^{a}$ | $14.9^{b}$ | $11.9^{b}$ | 2.91 | 3.50 | 4.12 | <0.001 | 0.919 | <0.001 | 0.020 | 0.587 | 0.674 |
| | Standing | 11.6 | 19.4 | 14.3 | 15.8 | $28.9^{a}$ | $11.4^{b}$ | $10.3^{b}$ | 2.07 | 2.79 | 3.01 | 0.012 | 0.754 | <0.001 | 0.076 | 0.597 | 0.947 |
| | Resting | 7.97 | 9.03 | 7.19 | 10.0 | 8.72 | 9.03 | 7.76 | 2.17 | 2.09 | 2.32 | 0.494 | 0.004 | 0.753 | 0.482 | <0.001 | 0.096 |
| | Outside | 0.71 | 0.71 | 0.55 | 0.91 | - | $1.71^{a}$ | $0.30^{b}$ | 0.41 | 0.37 | 0.64 | 1.00 | 0.568 | 0.003 | <0.001 | 0.546 | 0.972 |
| During live larvae provision | | | | | | | | | | | | | | | | | |
| FRB | Ground p. | 5.98 | 9.27 | 7.01 | 7.92 | 6.63 | 7.10 | 8.77 | 0.70 | 0.73 | 1.15 | 0.009 | 0.478 | 0.320 | 0.054 | 0.049 | <0.001 |
| | Object p. | 3.89 | 0.91 | 1.61 | 2.19 | $1.84^{ab}$ | $1.12^{b}$ | $3.19^{a}$ | 0.61 | 0.69 | 0.55 | 0.016 | 0.663 | <0.001 | 0.042 | <0.001 | 0.029 |
| | Scratching | 1.00 | 1.26 | 1.35 | 0.93 | $1.47^{ab}$ | $0.63^{b}$ | $1.53^{a}$ | 0.22 | 0.13 | 0.41 | 0.463 | <0.001 | <0.001 | 0.010 | <0.001 | 0.006 |
| | Eating larvae | - | $13.5^{\dagger}$ | 14.4 | 12.6 | - | $14.5^{a}$ | $12.4^{b}$ | - | 1.21 | 0.96 | - | 0.401 | 0.050 | - | - | 0.304 |
| CB | Preening | 2.09 | 2.02 | 2.39 | 1.77 | $1.19^{b}$ | $3.56^{a}$ | $2.05^{b}$ | 0.28 | 0.27 | 0.48 | 0.886 | 0.179 | 0.002 | 0.562 | 0.100 | 0.351 |
| AB | Walking | 22.7 | 27.7 | 24.1 | 26.1 | 26.7 | 25.8 | 23.0 | 1.88 | 3.04 | 4.13 | 0.113 | 0.729 | 0.456 | 0.211 | 0.852 | 0.006 |
| | Standing | 15.9 | 16.1 | 14.3 | 17.8 | $23.8^{a}$ | $17.1^{a}$ | $10.0^{b}$ | 1.31 | 1.70 | 2.87 | 0.926 | 0.223 | 0.007 | 0.007 | 0.710 | 0.889 |
| | Resting | 6.90 | 7.95 | 5.28 | 10.4 | $3.49^{c}$ | $12.8^{a}$ | $9.11^{b}$ | 1.17 | 0.40 | 1.02 | 0.650 | <0.001 | <0.001 | 0.971 | <0.001 | 0.324 |
| | Outside | 3.64 | 1.82 | 4.16 | 1.62 | - | 2.66 | 2.54 | 0.73 | 1.03 | 0.82 | 0.129 | 0.139 | 0.940 | 0.904 | 0.001 | 0.102 |
| During the afternoon | | | | | | | | | | | | | | | | | |
| FRB | Ground p. | 5.21 | 6.62 | 5.93 | 5.81 | 7.63 | 4.98 | 5.33 | 0.76 | 0.43 | 0.97 | 0.341 | 0.871 | 0.083 | 0.001 | 0.215 | <0.001 |
| | Object p. | 1.17 | 1.14 | 1.28 | 1.04 | $3.44^{a}$ | $0.65^{b}$ | $0.69^{b}$ | 0.22 | 0.21 | 0.40 | 0.935 | 0.543 | <0.001 | 0.926 | <0.001 | 0.427 |
| | Scratching | 0.93 | 1.87 | 2.01 | 0.87 | $3.72^{a}$ | $0.54^{b}$ | $1.16^{b}$ | 0.33 | 0.32 | 0.35 | <0.001 | <0.001 | <0.001 | 0.285 | <0.001 | <0.001 |
| CB | Preening | 3.41 | 3.35 | 4.07 | 2.81 | 2.81 | 3.76 | 3.66 | 0.37 | 0.47 | 0.63 | 0.866 | 0.047 | 0.446 | 0.149 | 0.571 | 0.899 |
| AB | Walking | 17.7 | 18.86 | 19.2 | 17.4 | $34.3^{a}$ | $20.0^{b}$ | $8.89^{c}$ | 1.92 | 2.75 | 3.11 | 0.748 | 0.736 | <0.001 | 0.013 | 0.326 | 0.100 |
| | Standing | 14.5 | 16.01 | 14.9 | 15.6 | $25.3^{a}$ | $16.6^{b}$ | $8.40^{b}$ | 1.22 | 2.21 | 1.78 | 0.059 | 0.850 | <0.001 | 0.121 | <0.001 | 0.537 |
| | Resting | 6.37 | 8.19 | 7.39 | 7.06 | $2.46^{c}$ | $14.6^{a}$ | $10.5^{b}$ | 1.12 | 1.03 | 0.98 | 0.128 | 0.695 | 0.003 | 0.312 | 0.316 | 0.022 |
| | Outside | 1.85 | 1.17 | 1.79 | 1.21 | - | $2.12^{a}$ | $1.03^{b}$ | 0.52 | 0.43 | 0.46 | 0.334 | 0.195 | 0.004 | <0.001 | <0.001 | 0.347 |

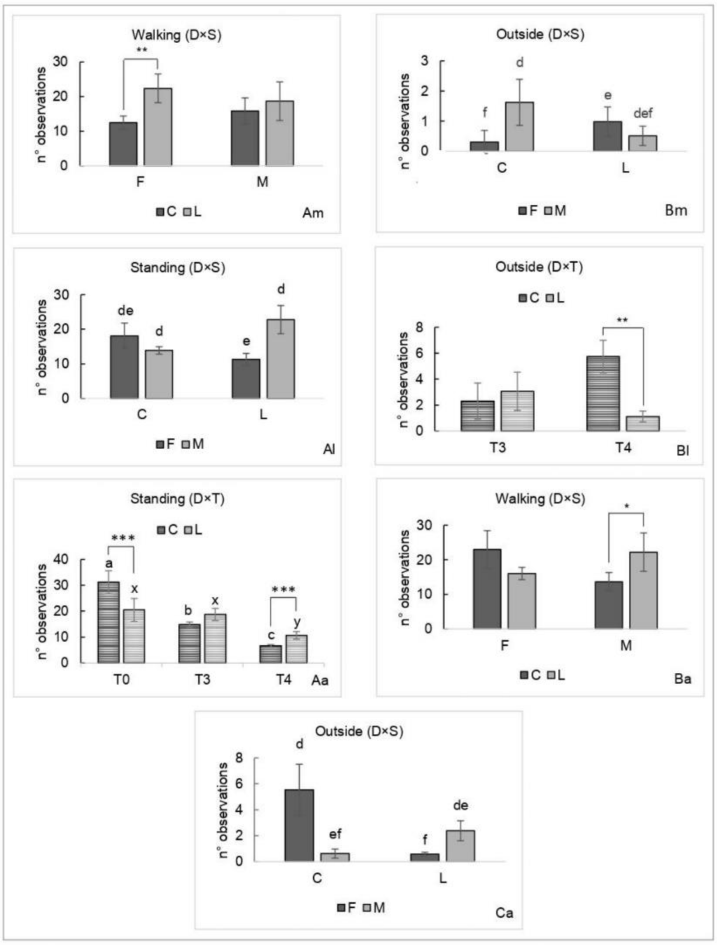
Abbreviations: diet×time (D×T), diet×sex (D×S), and sex×time (S×T); FRB, Foraging-related behaviour; CB, Comfort Behaviour; AB, Activity Behaviour; C, control groups; L, groups supplemented with live black soldier fly larvae; T0, time 0; T3, time 3; T4, time 4; p, pecking. †Arithmetic mean of both sex and times. The superscript letters a,b,c indicate significant differences at $P \le 0.05$.

Figure 1. Effects of the live black soldier fly provision on the foraging related behaviour performed by both males (M) and females (F) of the medium growing hybrid label naked neck chicken, in relation to the interactions between diet (D), sex (S), time (T), in the morning (m), during BSFL provision (l), and in the afternoon (a) (n=4).




Abbreviations: diet×time (D×T), diet×sex (D×S), and sex×time (S×T); T0, time 0; T1, time 1; T2, time 2; C, control groups; L, groups supplemented with live black soldier fly larvae. The letters A, B, C, D, E are reported to uniquely identify each graph. *indicates significant differences within the times at P ≤ 0.05. **indicates significant differences within the times at P<0.01; ***indicates differences within the times at P<0.001. The superscript letters a,b,c indicate significant differences between the female groups or control groups at different times; the letters x,y,z indicate significant differences between the male groups or groups supplemented with live black soldier fly larvae at different times; the letters d,e,f indicate differences among the CF, LF, CM and LM groups at P≤0.05.

Figure 2. Effects of the live black soldier fly provision on the activity behaviour performed by males (M) and females (F) of the medium growing hybrid label naked neck chicken, in relation to the interactions between diet (D), sex (S), time (T), in the morning (m), during BSFL provision (l), and in the afternoon (a) (n=4).



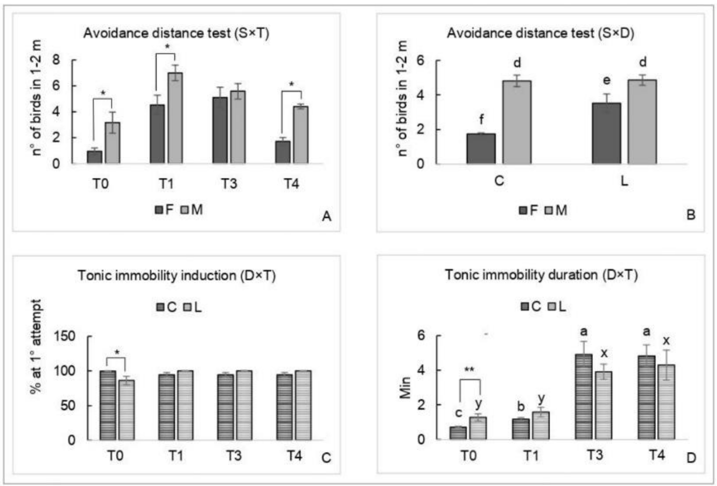

Abbreviations: diet×time (D×T), diet×sex (D×S), and sex×time (S×T); T0, time 0; T1, time 1; T2, time 2; C, control groups; L, groups supplemented with live black soldier fly larvae. The letters A, B, C are reported to uniquely identify each graph. *indicates significant differences within the times at $P \leq 0.05$. **indicates significant differences within the times at $P<0.01$; ***indicates differences within the times at $P<0.001$. The superscript letters a,b,c indicate significant differences between the female groups or control groups at different times; the letters x,y,z indicate significant differences between the male groups or groups supplemented with live black soldier fly larvae at different times; the letters d,e,f indicate differences among the CF, LF, CM and LM groups at $P \leq 0.05$.

Table 3. Effects of the live black soldier fly provision on the ethological tests and animal-based welfare measurements performed on males (M) and females (F) of the medium growing hybrid label naked neck chicken, in relation to diet (D), sex (S), time (T), and their interactions (n=6).

| Items | D | | S | | T | | | | | SEM | | | P-value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | L | F | M | T0 | T1 | T2 | T3 | T4 | D | S | T | D | S | T | D×S | D×T | S×T |
| AD test, birds in 1-2m | 2.90 | 4.13 | 2.48 | 4.83 | 1.74$^{c}$ | 5.63$^{a}$ | - | 5.34$^{a}$ | 2.75$^{b}$ | 0.25 | 0.16 | 0.48 | 0.003 | <0.001 | <0.001 | <0.001 | 0.982 | <0.001 |
| TII at first attempt, % | 95.6 | 96.4 | 98.6 | 93.4 | 92.7$^{b}$ | 97.1$^{ab}$ | - | 97.2$^{a}$ | 97.1$^{b}$ | 1.78 | 1.89 | 2.14 | 0.649 | 0.076 | 0.025 | 0.141 | 0.022 | 0.961 |
| TID, min | 2.11 | 2.41 | 2.18 | 2.33 | 0.96$^{c}$ | 1.36$^{b}$ | - | 4.38$^{a}$ | 4.55$^{a}$ | 0.17 | 0.15 | 0.25 | 0.336 | 0.571 | <0.001 | 0.215 | <0.001 | 0.025 |
| ECM, ng/g | 38.6 | 38.1 | 36.6 | 40.1 | 46.0$^{a}$ | 38.9$^{b}$ | - | - | 31.5$^{c}$ | 1.26 | 1.21 | 1.78 | 0.662 | <0.001 | <0.001 | 0.219 | 0.098 | 0.413 |
| H/L ratio | 0.91 | 1.27 | 1.06 | 1.10 | - | - | - | - | - | 0.13 | 0.13 | - | 0.050 | 0.816 | - | 0.722 | - | - |

Abbreviations: C, control groups; L, groups supplemented with live black soldier fly larvae; T0, time 0; T1, time 1; T2, time 2; T3, time 3; T4, time 4; diet×time (D×T), diet×sex (D×S), and sex×time (S×T); AD, avoidance distance test; TI; tonic immobility; TID, tonic immobility duration; ECM, excreta corticosterone metabolites; H/L, heterophile/lymphocyte ratio. The superscript letters a,b,c indicate significant differences at $P \leq 0.05$.

Figure 3. Effects of the live black soldier fly provision on the ethological tests performed by males (M) and females (F) of the medium growing hybrid label naked neck chicken, in relation to the interactions between diet (D), sex (S), and time (T) (n=6).




Abbreviations: diet×time (D×T), diet×sex (D×S), and sex×time (S×T); T0, time 0; T1, time 1; T2, time 2; T3, time 3; C, control groups; L, groups supplemented with live black soldier fly larvae. The letters A, B, C, D are reported to uniquely identify each graph. *Indicates significant differences within the groups at P≤0.05; **indicates significant differences within the groups at P≤0.01. The superscript letters a,b,c indicate significant differences between female groups or control groups at different times; the letters x,y,z indicate significant differences between male groups or groups supplemented with live black soldier fly larvae at different times; the letters d,e,f indicate differences among the CF, LF, CM and LM groups at P<0.05 and F and M groups over time.

## 11 Overall discussion

In the following chapter, an overall discussion about the studies included in this thesis will be provided, in order to offer a comprehensible overview of the PhD objectives. Moreover, tools to understand the prominence of the local breeds' conservation will be explicated, clarifying the strategies to maintain them on trend in a reality that leaves scant space for these niche productions. Finally, the benefits in the use of BSFL as feed and environmental enrichment on growth and slaughter performance, welfare, and meat quality will be emphasized.

Nowadays, the consumers' attitude towards meat is changing due to the elevate environmental impact derived from its production. The major concerns are mostly directed to the global warming, which sees the meat sector as an important contributor to the issue. The current world development and the increasing meat request is attributed to developing countries, such as Africa, Asia, Pacific regions, and Latin American region, covering the 60% of the total meat demand in 2030 (OECD-FAO Agricultural Outlook 2021-2030, 2021). The poultry, and especially, the chicken meat, would represent the first protein source in those world areas, for the same reasons it became the most consumed meat in developed countries: low production cost and rearing cycle, protein quality source, and absence of cultural barriers (EC, 2018; OECD-FAO, 2019). However, developed countries, such as the North America, have been predicted to contribute for about 10% of the increase. Different projections have been made in other developed countries. For instance, the meat consumption increase predicted in Europe is close to 1% in 2030 (OECD-FAO Agricultural Outlook 2021-2030, 2021). Such differences in meat consumption in developed countries are surely related to the population awareness about the dietary habit impact. Although the percentage of people who already choose a more sustainable approach, its adoption remains currently limited. A study involving 34 papers devised a detailed eco-friendly profile (Sanchez-Sabate & Sabaté, 2019), which corresponds to young non-vegan/vegetarian women, more probably from European or Asiatic countries than U.S., who reduced the amount of meat assumed as ecological approach. Moreover, such research demonstrated a change in the willingness of the consumers, currently oriented to stop or minimize the amount of meat consumed (Sanchez-Sabate & Sabaté, 2019). However, it must be underlined that the culinary education represents a delicate topic, being difficult to modulate consumers' choices and tastes. Considering the sustainability, the chicken meat should be devised as the favored one, being less impactful than the other animal products (Leinonen & Kyriazakis, 2016). Thus, the promotion of local genotypes represents the logical prosecution, favoring the quality over the quantity and safeguarding, as a natural consequence, the biodiversity heritage presents on the territory (Cendron et al., 2020). Moreover, as Zanetti et al., (2010) and Cendron et al. (2020) reported, the intensive rearing systems radically interfered in the biodiversity structure, threatening the autochthonous genotypes subsistence. Considering the next steps in poultry production, such biodiversity might be necessary for the next chickens' generations, in order to cope with future challenges (e.g., resistance and adaptability to climate changes and diseases). Nonetheless, formation and information campaigns must be at the basis even of future measures in regard to this topic, aiming at increasing the awareness of the consumer in terms of environmental impact and biodiversity tutelage (Cendron et al., 2020). From the research point of view, it is extremely difficult to completely safeguard the livestock species worldwide, considering even the absence of fundings (Zhao et al., 2021), making thus necessary a rational selection based on the population structure, conservation priority, and runs of homozygosity is the only solution (Gao et al., 2023). Undeniably, a responsible consume of meat, rather than a complete withdrawal, in favor of local breeds instead of fast-growing broilers, would support the maintenance of a connection with the market, being the major number of breeds reared and retrieved as egg and meat producers. Moreover, it must be stated that the local chicken breeds' products were surely more expensive than the commercial ones, but comparable to the ones obtained by niche poultry as the Poulet de Bresse (in France) or from organic systems (reference). Such information represents the evidence of real niche creation opportunity in the market in support to the biodiversity (Franzoni et al., 2021)

In our first trial, differences are reported not only between the studied genotypes BP and BS, but also in terms of slaughter age. Overall, the study aimed at providing useful information for the breeders, to be able to select the most efficient slaughter time compatibly with their necessities. The crucial point lies in the balance between the obtainment of an appreciated good quality product and the efficiency of the breed itself, in an effort to sustain the farmers and, as a consequence, the breed.

The main results of our first experiment, in terms of yield at slaughter, showed a significant effect of the breed on the carcass yield at 5, 7 and 8 months of age ($P<0.05$), resulting in a better performance of the BP breed on the BS one. The carcass yield values corresponded to 60.28%, 58.53%, 58.44%, and 55.88% for BPM, BPF, BSM, and BSF, respectively. Moreover, the BPM and BSM and F showed higher values in breast yield at 7 and 8 months of age ($P<0.01$) and generally F displayed higher values than M ($P<0.01$). The opposite has been observed for the thigh yield, where the M displayed higher values than F ($P<0.01$). Differences among meat quality attribute have been even observed in BP and BS, presenting the M a higher redness of breast, whereas the opposite was observed for both breast meat b* and L* ($P<0.001$). Regarding the meat proximate composition, the moisture and CP values were higher in the M than the F (74.26% *vs* 73.37%, and 25.31 *vs* 24.79, respectively; $P<0.01$), and a major moisture level was detected in animals slaughtered at 5 than 7 or 8 months ($P<0.01$), reflecting possible differences in meat juiciness. Relevant differences were noted in the breast CF content, lower in M than F (1.75% *vs* 4.4%; P<0.01), and the fatty acids composition differed between M and F in a similar way in both breast and thigh. Particularly, the SFA were predominant in M compared to F (48.79% *vs* 46.54; P<0.05) as well as the PUFA (27.93 *vs* 10.18; P<0.001), while the MUFA were more abundant in F than M (34.30 *vs* 40.69; P<0.001). Numerical differences have been recorded even in the fatty acid composition of the breast and the thigh in the BP and BS, being the PUFA higher in the breast (19.94%) than the tight (4.20%), despite no statistical analysis have been performed on these parameters. A comparison of our results with other studies is provided in the following tables.

Table X. Slaughter performance of various poultry genotype.

| Poultry | Sex | Age (d) | SW (g) | Slaughter yield (%BW) | Breast yield (%CC) | Tight yield (%CC) | Reference |
|---|---|---|---|---|---|---|---|
| **Fast growing hybrids** | | | | | | | |
| Vencobb | M+F | 36 | 2000 | 74.2 | 38.0 | 19.1 | Devatkal et al., 2019 |
| Ross-308 | M+F | 38 | 2010 | 76.5 | 35.3 | 38.1 | Canoğulları Doğan et al., 2019 |
| **Medium growing hybrids** | | | | | | | |
| Bresse | - | 112 | 1520 | 63.6 | 14.5 | 20.4 | Jaturasitha et al., 2008 |
| Rhode Island Red | - | 112 | 1580 | 64.4 | 11.7 | 19.3 | Jaturasitha et al., 2008 |
| T2-Y2 | M+F | 72 | 1974 | 75.6 | 24.6 | 41.1 | Canoğulları Doğan et al., 2019 |
| Inbro | M+F | 50 | 2000 | 68.6 | 30.0 | 17.5 | Devatkal et al., 2019 |
| **Slow growing hybrids** | | | | | | | |
| *Bionda Piemontese* | *M+F* *1°s* | *5 months* | *3420* | *60.6* | *17.6* | *36.1* | *Chapter8* |
| | *2°s* | *6 months* | *1947* | *60.0* | *16.4* | *35.8* | |
| | *3°s* | *7 months* | *2121* | *59.6* | *18.2* | *35.3* | |
| | *4°s* | *8 months* | *2243* | *57.9* | *19.5* | *36.2* | |
| *Bianca di Saluzzo* | *M+F* *1°s* | *5 months* | *1701* | *58.5* | *18.4* | *35.6* | *Chapter 8* |
| | *2°s* | *6 months* | *1978* | *58.5* | *16.3* | *35.2* | |
| | *3°s* | *7 months* | *2200* | *56.7* | *18.7* | *35.6* | |
| | *4°s* | *8 months* | *2254* | *54.97* | *19.3* | *36.2* | |
| Mugellese | F | 140 | 772 | 77.80 | - | - | Mannelli et al., 2023 |
| Milanino | M+F | 235 | 2761 | 64.39 | - | - | Cerolini et al., 2019 |
| Thai native | - | 112 | 1280 | 65.9 | 15.5 | 19.6 | Jaturasitha et al., 2008 |
| Black-boned | - | 112 | 1100 | 63.7 | 12.5 | 20.06 | Jaturasitha et al., 2008 |

Abbreviations: d, days; SW, slaughter weight; CC, cold carcass; M, male; F, female; -, not reported; s, slaughter; *Ch.,* chapter.

TableX. Breast meat quality of various poultry genotypes.

| Poultry | Sex | Age (d) | L* | a* | b* | pH | Moi | CP | CF | SFA | MUFA | PUFA | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fast growing hybrids** | | | | | | | | | | | | | |
| - | M | 40 | - | - | - | - | 72.6 | 18.5 | 7.28 | - | - | - | Dalle Zotte et al., 2020 |
| Ross 308 | F | 37 | 46.4 | 1.64 | 8.80 | 5.86 | - | - | - | - | - | - | Weng et al., 2022 |
| Vencobb | M+F | 36 | 44.7 | 3.34 | 6.74 | - | - | - | - | 0.48 | 0.72 | 0.30 | Devatkal et al., 2019 |
| **Medium growing hybrids** | | | | | | | | | | | | | |
| Indbro | M+F | 50 | 51.42 | 1.56 | 4.27 | - | - | - | - | 0.28 | 0.28 | 0.17 | Devatkal et al., 2019 |
| **Slow growing hybrids** | | | | | | | | | | | | | |
| Bresse | - | 112 | 54.8 | 2.98 | 8.4 | 5.88 | 73.3 | 23.6 | 0.76 | 38.7 | 30.3 | 31.0 | Jaturasitha et al., 2008 |
| Rhode Island Red | - | 112 | 61.6 | 0.60 | 14.1 | 5.86 | 73.7 | 24.8 | 0.72 | 36.7 | 29.3 | 34.1 | Jaturasitha et al., 2008 |
| Xueshan | F | 101 | 46.4 | 2.38 | 13.8 | 6.07 | - | - | - | - | - | - | Weng et al., 2022 |
| *Bionda Piemontese* | *M+F* | *1°s 5 months* | *52.8* | *0.94* | *8.59* | *6.16* | *74.2* | *25.2* | *0.49* | *30.5* | *30.5* | *25.5* | *Chapter 8* |
| | | *2°s 6 months* | *51.6* | *0.78* | *9.50* | *6.22* | *74.4* | *24.4* | *0.46* | *32.2* | *32.2* | *23.0* | |
| | | *3°s 7 months* | *54.0* | *1.09* | *9.21* | *6.34* | *73.3* | *25.0* | *1.03* | *38.0* | *38.0* | *11.6* | |
| | | *4°s 8 months* | *51.8* | *2.92* | *10.7* | *6.23* | *73.6* | *24.9* | *0.44* | *36.2* | *36.2* | *16.5* | |
| *Bianca di Saluzzo* | *M+F* | *1°s 5 months* | *53.1* | *1.13* | *7.68* | *6.25* | *74.2* | *25.9* | *0.33* | *28.6* | *28.6* | *27.3* | *Chapter 8* |
| | | *2°s 6 months* | *52.7* | *0.20* | *7.99* | *6.23* | *74.1* | *24.4* | *0.59* | *33.6* | *33.6* | *21.3* | |
| | | *3°s 7 months* | *53.8* | *0.93* | *9.61* | *6.22* | *73.3* | *25.4* | *0.76* | *38.7* | *38.6* | *16.3* | |
| | | *4°s 8 months* | *51.0* | *2.40* | *9.65* | *6.33* | *73.6* | *25.0* | *0.56* | *33.4* | *33.4* | *18.0* | |
| Milanino | M+F | 235 | 52.30 | 2.47 | 4.57 | - | 72.20 | 25.20 | 1.26 | 40.42 | 30.04 | 29.54 | Ceroli- et al., 2019 |
| Polverara | M | 180 | - | - | - | - | 73.7 | 21.5 | 2.25 | - | - | - | Dalle Zotte et al., 2020 |
| Padovana | M+F | 183 | - | - | - | - | 74.2 | 17.9 | 6.63 | 39.3 | 25.6 | 33.8 | Dalle Zotte et al., 2019 |
| Thai native | - | 112 | 54.9 | 1.27 | 13.6 | 5.77 | 72.9 | 24.7 | 0.51 | 38.23 | 29.08 | 32.69 | Jaturasitha et al., 2008 |
| Black-boned | - | 112 | 50.7 | 1.66 | 10.5 | 5.88 | 72.1 | 24.4 | 0.53 | 35.41 | 28.78 | 35.81 | Jaturasitha et al., 2008 |
| Baicheng-You | M+F | 120 | 68.26 | - | - | 6.03 | 72.93 | 30.24 | - | - | - | - | Sarsenbek et al., 2013 |

Abbreviations: d, days; L*, lightness; r*, redness; b*, yellowness; Moi, moisture ; CP, crude protein; CF, crude fat; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; M, male; F, female; -, not reported; d; BP, Bionda Piemontese; BS, Bianca di Saluzzo; s, slaughter.

As it can be understood from the previous examples, several difference could characterize a local breed, and infinite is the potential of each sensorial attribute. The age of slaughter of the birds can vary from a couple of months until 7-8 months of age, as in our research. By contrast, the carcass, breast and thigh yield are fundamental contributors to the maintenance of local breeds, being directly related to the farmers' income, especially in developing country, where the food quantity rather than food quality still represents the first purchase driver, despite the cultural habits and local traditions widely leverage on the market demand of various countries. For instance, in African countries, several chicken strains present a reduced growth which leads to scant carcass yield, as underline in the review by Kpomasse et al. (2023). Thus, a selection of such breeds designed to improve their performance is required, to guarantee both the breed conservation with its attributes (e.g., high temperature resistance) and the farmers' income. The results reported in our study related to the carcass yield were few percentage points far (about 2-3%) from some medium-growing hybrids, despite wide differences can be observed among other local breeds, with differences in carcass yield of 10-15% (Table X); however, the differences in the age of slaughter must be taken into account. A similar consideration can be given to the meat quality parameters that can be observed and appreciate by the consumers. For instance, similar values of breast L* index within the slow-growing genotypes were depicted, while wide variations for a* index were recorded, from a minimum of 0.40 for our BS to about 1.7 for a Thailandese local breed. The meat b* index varied instead from about 4.3 to 14.1 within all the slaughter types included. However, considering the developed areas of the world, the sustainability key should be the meat consumption reduction, sponsoring a moderate amount of quality food purchased, compatible with the population's economic availability, thus supporting small farms and autochthonous chicken products.

With a view to provide new tools to improve sustainability and welfare and alternative poultry farming, as the biological system employes slow and medium-growing birds, a second trials carried out with the aim of evaluating the use of insect larvae on chickens' performance, welfare, and meat quality, as reported in the chapter 9, 10, and 11.

Overall, the use of insects as livestock feed or environmental enrichment revealed a great potential (Gligorescu et al., 2020). Firstly, the natural presence of insects as poultry feed in nature cannot be ignored. Moreover, the insects' motility represents one of the key aspects for such success. By virtue of their motion properties the live BSFL attract chickens' interest more than any other inanimate environmental enrichment, being a constant stimulus for their explorative and foraging behaviors (Clara et al., 2009; Star et al., 2020). Furthermore, in modern commercial farms, included the slow and medium-growing rearing systems, the birds' possibilities in availing live larvae naturally is improbable, making the live BSFL supplementation a valid tool to overcome this lack (Riber et al., 2018; Star et al., 2020).

Various studies have been carried out on the live BSFL as well, with positive effects or either way no interferences reported on growth and slaughter performance, and blood traits of broiler chickens and turkey poults (Bellezza Oddon et al., 2021; Ipema et al., 2020a; Ipema et al., 2020b; Ipema et al., 2022; Veldkamp & van Niekerk, 2019). No information was instead available on medium-growing hybrids. Thus, our second trial connects the sustainability concepts described so far in this thesis, resumed, with supportive literature in the table below.

Table X. Effects of the live black soldier fly larvae provision to different poultry species.

| Poultry | Amount (%) | Frequency | Method | Effects on supplemented groups | | | | References |
|---|---|---|---|---|---|---|---|---|
| | | | | Growth performance | Slaughter performance | Blood parameters | LCT | |
| **FGH** | 10 | 1/day | Plates | Not dected**e**d | Higher spleen RW | Not detected | Increase | Bellezza Oddon et al., 2021 |
| | 5 or 10 | 4 or 7 times/d | Scattered or tubes with holes | Not decteded | Not decteded | - | - | Ipema et al., 2020 |
| ***MGH*** | *10* | *1/day* | *Plates* | *Higher BW* | *Higher spleen RW* | *Trend in cholesterol reduction; lower GGT* | *Reduction* | *Chapter 9* |
| **Turkeys** | 10 | 1/day | Feeders | Hgher DFI, BWG, LW, lower FCR | - | - | Decrease | Veldkamp & van Niekerk, 2019 |

Abbreviations: FGH, fast growing hybrid; MGH: medium growing hybrid; d, day; RW, relative weight; BW, body weight; DFI, daily feed intake; BWG, body weight gain; FCR, feed conversion ratio; GGT, gamma-glutamyltransferase; LCT, larvae consumption time.

Further research is surely necessary in this field, to investigate how the live larvae can impact on the growth and slaughter performance of slow-growing genotypes, in which rearing systems the live larvae could apport beneficial effects on their health and welfare, without unpaired the performance of the birds or in case improve them. To conclude, it would be an utopistic statement saying that a total conversion of consumers' habits and rearing system realities will be obtainable in the brief period, especially considering the constant population increase and the current modernization of developing countries (OECD-FAO Agricultural Outlook 2021-2030, 2021). Therefore, the insect supplementation in chickens' diet, by virtue of their protein composition, represents a valid and sustainable alternative to conventional protein source, being advantageous from an environmental point of view, without neglect the supporting role in improving social condition and livelihood of developing countries populations, in which however, the insects have often been considered as food and feed since antiquity (van Huis et al., 2013).

Moving forward, the live BSFL not only represent a feed ingredient, but even an environmental enrichment, by virtue of larvae's motion ability which is the matter of interest for chickens (Clara et al., 2009; Star et al., 2020). The choice of a medium-growing hybrid reared in our second study has been dictated both by the lack of information on this kind of genotypes and by the underestimated welfare conditions in which these animals often live. In fact, they have been ideated to be adaptable to organic and free-range rearing systems, but often raised in sub-optimal conditions (Van De Weerd et al., 2009; Riber et al., 2018). Various studies reported the effectiveness of live BSFL in improving chickens' welfare, with better plumage and skin damage score than the control groups and a reduction in the aggressive behaviors in turkeys poults and broiler chickens (Veldkamp & van Niekerk, 2019; Star et al., 2020; Ipema et al., 2022), despite no differences were instead identified in our study. An amelioration of leg health and birds activity, in relation to the larvae provision frequency was observed in broiler chickens (Ipema et al., 2020b), and a fulfilment of explorative behavioral needs in laying hens (Star et al., 2020). Similarly, both physical activity and explorative behaviors were enhanced in our research, the latter especially in females. In addition, a general implemented foraging behavior (Ipema et al., 2020a; Biasato et al., 2022) and a reduction of fear of the birds was observed (Ipema et al., 2020a), corroborating our results obtained with both video-recordings and acoidance distance test. Although the organic and free-range rearing systems should imply better living conditions than the conventional systems, the welfare of such birds is often taken for granted, while more attention and dedication in terms of environmental enrichment and birds' management should be ensured (Van De Weerd et al., 2009; Riber et al., 2018). In the Table X the welfare results obtained from our second trial, compared with the available literature.

The presented results corroborate the potential of live BSFL use as environmental enrichment, demonstrating outstanding results on birds foraging and explorative behaviors and the maintenance of physical activity during aging, moment in which the sedentarism of such birds tend to decrease (Bokkers & Koene, 2003; Jacobs et al., 2021). Moreover, the higher efficacy of scattering live larvae compared to dried or live ones administered by feeders (Ipema et al., 2022), routes future research to this kind of provision even for this hybrid, despite the distribution during the day and the entire life-cycle remained so far unknown and necessitate further investigations. Overall, these conditions effectively satisfy the birds' welfare and could meet the consumer's expectation. However, since questionnaires or records on consumers' perception have not been included in this research, further data should be collected to provide a response. A better understanding of the consumers' opinion and attitude in purchasing products derived by insect-fed chickens represets a fundamental step. Such way, new tools would be accessible to face and overcome the challenges related to the public acceptance of insects as animal feed, which are endowed of valuable benefits for environment and animal welfare.

Table X. Live black soldier fly larvae provision on poultry welfare and behavior.

| Poultry | Quantity (% DFI) | Frequency | Method | Effects | | | | References |
|---|---|---|---|---|---|---|---|---|
| | | | | Behavior | Fear | Leg health | Plumage status | |
| **FGH** | 5 or 10 | 4 or 7 times/d | Scattered or tubes with holes | Increased activity and foraging in relation to the frequency and method of distribution | Reduced in relation to the frequency and method of distribution | Not detected | - | Ipema et al., 2020a |
| | 5 or 10 | 2 or 4 times/day | Scattered | Increased activity and foraging, especially at the highest quantity and frequency | - | Improved especially at the highest quantity and frequency; | - | Ipema et al., 2020b |
| | 8 | 4 times/day | Scattered | Increased activity and foraging | - | Improved | - | Ipema et al., 2022 |
| | 10 | 1/day | Plates | Increased activity and foraging, wing flapping, and stretching | - | Not detected | Not detected | Biasato et al., 2022 |
| ***MGH*** | *10* | *1/day* | *Plates* | *Increased in explorative, foraging and standing frequency;* | *Reduced* | *Not detected* | *Not detected* | *Chapter 9* |
| **LH** | 10 | Within 6 hours | Dispenser | Increased number of BSFLbirds on the litter in the morning hours → exploration stimulation | - | - | Improved | Star et al., 2020 |
| | 10,20, *ad libitum* | 1/day | feed bowls/troughs | Not detected | - | - | Not detected | Tahamtani et al., 2021 |
| **Turkeys** | 10 | 1/day | Feeders | Increased foraging during the first WOA, lower tendency in such behaviors in at 3-5 WOA; Reduced pecking at back and tail base at 5 WOA. | - | - | Improvement tendency in BSFL groups | Veldkamp and van Niekerk, 2019 |

FGH, fast-growing hybrid; MGH: medium growing hybrid; LH, laying hens; WOA, weeks of age; BSF, black soldier fly larvae.

## 12 Overall conclusions

To provide an overall conclusion, the poultry production sustainability represents surely a complicated and faceted topic. However, efficient and effective approaches can be adopted to promote it. The valorization of local breeds could be a valid tool to support a sustainable and responsible meat consumption, sustaining, at the same time, the biodiversity. The steps to accomplish this aim encompass the characterization of breeds' performance and meat quality. The growth and slaughter performance of local breeds, such as BP and BS, can vary among genotypes and can be improved by means of genetic selection, but the meat composition and the meat quality attributes require to be valorized, in order to make the product recognizable and secure to these animal products a stable market niche. The objective of this research is to provide valuable and spendable information for the breeders. Additionally, the farmers' income must be considered, despite we did not evaluate in this study the economic aspects related to such productions. Nonetheless, this aspect that can be sustained by the research, for instance, providing information on the best slaughter age and underline the main meat characteristics that could affect the consumers' appreciation and purchase. Thus, we overall identified the most suitable period for the slaughter at 7 months of age based on the cold carcass weight, which remained unvaried from that moment on, and the distinct red and yellow meat coloration of males and females, respectively, which characterized the final product. Furthermore, amelioration in the sustainability can be apported by favoring of organic and free-range rearing systems, in which slow and medium-growing genotypes are preferred compared to the fast-growing commercial lines, especially if combined with alternative feeding strategy. On this purpose, the use of insects revealed to be an intriguingly means to achieve this goal even in medium-growing chickens. In particular, the inclusion of 10% BSF live larvae in chickens' diet showed beneficial effects on the growth and slaughter performance as well as the health blood parameters with no compromission of the quality attributes and *in vitro* protein digestibility of meat. It must be underlined that in our study we were able to appreciate improvements in the performance and welfare of birds despite the optimal management and welfare conditions usually not as good in big farms, underlying the potential of live BSFL administration. Since the BSF can be satisfyingly reared on food-waste in a circular economy context, the costs for the supplementation can be compensated and the profit of farmers can be guarantee. Finally, advantages have been observed even on the birds' welfare, including in our study a reduction of females fear towards the operator, which means improved welfare of the animals and management practice, by virtue of the better relationship bird-operator than in the control groups. Finally, the implementation of exploratory and foraging frequency demonstrated the contribution in the exploitation of birds' natural behavioral repertoire and in the maintenance of the animal's health and active till the end of the cycle. To conclude, this thesis encompasses the themes of the biodiversity conservation and insects as feed and environmental enrichment, which constitute high value tools to cope with the world challenges nowadays, related especially to climate changes and the necessity of alternative and more sustainable protein sources. Moreover, this work represents even a fundamental contribution in the support and improvement of chicken welfare, filling the knowledge gap on the effect of live BSFL in medium growing chickens.

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