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COMPARISON BETWEEN THE DIGESTIBILITY OF CLASSICAL PROTEIN SOURCES AND INSECT MEAL IN IN VITRO ANIMAL MODELS

CONFRONTO TRA LA DIGERIBILITÀ DI FONTI PROTEICHE CLASSICHE E DELLA FARINA DI INSETTO IN MODELLI ANIMALI IN VITRO

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ABSTRACT

The projected increase in the demand for animal products will require the use of highly digestible protein sources in animal nutrition to improve protein utilisation, reduce nitrogen losses and mitigate the environmental impact of livestock production. Insect meals have emerged as promising alternative protein sources, although their digestibility remains to be fully assessed in comparison with established feed ingredients. In this study, the digestibility of black soldier fly (*Hermetia illucens*) larvae meal was compared with that of roasted full-fat soybean, whole sunflower seed and herring meal in cattle, pigs and poultry. After a characterisation of their chemical composition, the four feed ingredients were submitted to in vitro digestion methods specific to each animal, and their dry matter and crude protein digestibility were measured. Crude protein digestibility of the insect meal was 79.1, 63.4 and 49.3% in cattle, pigs and poultry, respectively. In all three species, these values were comparable to those of herring meal, but lower than those of soybean in all species and of sunflower seed in cattle and poultry. This lower digestibility was attributed to the chitin of the larval exoskeleton, which can bind part of the protein, with a less pronounced effect in cattle, likely due to the partial degradation of chitin by the ruminal microbiota. Overall, black soldier fly larvae meal showed a digestibility comparable to that of herring meal across species, but generally lower than that of the plant oilseeds tested, suggesting a greater potential as an alternative to animal-derived than to highly digestible plant protein sources.

INTRODUCTION

The growth of the world population and the rise in per-capita income are increasing the demand for animal-source food, and satisfying this demand will require a considerable increase in animal production (Nardone et al., 2010). This expansion, however, cannot be pursued without considering its environmental consequences.

Among the environmental issues associated with livestock production, nitrogen losses represent a major concern. A substantial proportion of the nitrogen supplied through dietary protein is not retained by the animal but excreted and lost in the environment, and the faecal fraction of this excreted nitrogen is largely determined by the digestibility of dietary protein (Siegert et al., 2025). Because undigested protein is excreted rather than utilized, increasing protein digestibility represents one of the main nutritional strategies for reducing nitrogen losses. Beyond its environmental implications, protein digestibility also has an economic relevance, since protein is the most expensive component of animal diets and its efficient utilization depends on its digestibility (Lu et al., 2022).

In this regard, increasing attention has been given to the identification of alternative protein sources, an interest that is further supported by the fact that conventional protein sources commonly used in animal feeding compete with human food and raise sustainability concerns, since their production can contribute to the overexploitation of natural resources and to the loss of natural habitats (Biteau et al., 2026). Among the proposed alternatives, insects, particularly black soldier fly (*Hermetia illucens*) larvae, have emerged as one of the most promising protein sources for animal feeding (Shah et al., 2022).

Chapter 1: ENVIRONMENTAL SUSTAINABILITY OF LIVESTOCK PRODUCTION

1.1 Global demand for animal-source foods and the role of livestock production

The global population is projected to reach approximately 9.7 billion people by 2050, representing a 17% increase relative to current levels (United Nations, 2024). This demographic expansion will be concentrated predominantly in developing regions, where urbanization is also expected to accelerate substantially: projections indicate that, by 2050, approximately 67% of the world's population will live in urban areas (United Nations, 2025). As populations migrate to urban centers, access to higher-income employment opportunities expands, leading to increases in per capita income and driving profound transformations in dietary patterns and food consumption habits (FAO, 2009). Rising income levels are in fact closely associated with increased consumption of animal-source foods, as the diet shifts progressively from one based on starchy staples to a more diversified diet that also includes more meat and dairy (Delgado, 2003; Nardone et al., 2010; Godfray et al., 2018). Estimates indicate that global demand for all major animal-source food categories will rise substantially between 2000 and 2030, with projected increases of +170% for poultry meat, +97% for milk, +88% for mutton, +81% for beef, +70% for eggs and +66% for pork (Robinson and Pozzi, 2011). At per-capita level, global consumption of livestock products is projected to grow between 26% and 94% over the period 2000-2030 (Robinson and Pozzi, 2011). Meeting this growing demand will inevitably require a substantial increase in animal production (Nardone et al., 2010). However, this expansion cannot be pursued without

addressing the environmental consequences associated with intensive livestock farming.

1.2 Greenhouse gas emissions and nutrient losses from livestock production

The livestock sector is a major source of anthropogenic greenhouse gas (GHG) emissions, producing about 6.2 Gt CO_2eq per year and accounting for roughly 12% of total anthropogenic emissions (FAO, 2023). According to the same report, about 62% of livestock emissions come from cattle (around 3.8 Gt CO_2eq per year), while pigs represent 14%, chickens 9%, buffaloes 8% and small ruminants 7%. By commodity, the largest share of emissions comes from meat production (67%), followed by milk (30%) and eggs (3%). These emissions are classified as direct and indirect. Direct emissions – methane (CH_4) from enteric fermentation and methane and nitrous oxide (N_2O) from manure management – represent around 60% of the sector's total emissions, while indirect emissions, arising mainly from feed production, fertilizer and pesticide manufacture, land-use change and processing and transport – account for the remaining 40%. Considering the single gases, methane is the main greenhouse gas produced by the livestock sector, making up 54% (123 Mt CH_4) of total emissions, followed by carbon dioxide (CO_2) (31%; 1,960 Mt CO_2) and nitrous oxide (15%; 3.35 Mt N_2O) (FAO, 2023). Nitrous oxide is one of the chemical forms of nitrogen (N), an element that plays a central role in the environmental impact of the livestock sector (Uwizeye et al., 2020).

Crop-livestock production systems are the largest cause of anthropogenic alteration of the global nitrogen and phosphorus cycles (Bouwman et al., 2013). Animal manure is one of the main sources of phosphorus (P) inputs

to agricultural soils, and surplus P accumulates as residual soil P (Bouwman et al., 2013). This accumulated P can be lost through surface runoff and leaching to nearby water bodies, contributing to eutrophication and water quality degradation and favouring harmful algal blooms, hypoxia and fish kills (Jahan et al., 2025; Bouwman et al., 2013).

1.3 Nitrogen and the livestock sector

Nitrogen is an essential element for life, as it is a key component of amino acids, proteins and nucleic acids. The majority of nitrogen on Earth is found in the atmosphere as molecular nitrogen (N_2), which cannot be directly used by most organisms but must first be converted into a reactive form to become available (Galloway et al., 2021). Once it becomes reactive, nitrogen moves between soil, water and atmosphere, changing chemical form into ammonia (NH_3), nitrate (NO_3^-), nitrous oxide (N_2O) and other compounds, and producing a different environmental effect at each step (Galloway et al., 2021). Human activities, in particular the industrial production of fertilizers through the Haber-Bosch process and the cultivation of leguminous crops, have more than tripled the amount of reactive nitrogen created compared to natural terrestrial fluxes (Galloway et al., 2021). Assessments of the planetary boundaries identify the alteration of the nitrogen cycle as one of the limits that have been most severely exceeded: global nitrogen fixation has reached about 190 Tg N per year, roughly three times the proposed safe level of 62 Tg N per year (Richardson et al., 2023). Globally, the agri-food sector, particularly livestock, is one of the main contributors to this alteration, accounting for about one-third of anthropogenic reactive nitrogen emissions (Uwizeye et al., 2020).

1.3.1 Nitrogen use efficiency in farm animals

The large contribution of livestock sector to the alteration of nitrogen cycle is closely linked to how efficiently animals use the nitrogen they ingest. This efficiency is described by the nitrogen use efficiency (NUE), defined as the proportion of ingested nitrogen that is retained in products such as meat, milk and eggs; at the animal level it is expressed as grams of nitrogen in product per gram of nitrogen intake (Calsamiglia et al., 2010). In livestock this efficiency is generally low, so only a small part of the ingested nitrogen is retained, while most of it is excreted in urine and faeces and lost to the environment (Pomar et al., 2021). Because this excreted nitrogen is a source of environmental nitrogen losses, improving NUE is a central strategy to reduce the nitrogen footprint of animal production (Plomaritou et al., 2025). The portion of nitrogen that is not retained has two different origins: faeces and urine. Nitrogen from dietary protein that is not digested is excreted in the faeces, while nitrogen from amino acids that are absorbed but supplied in excess of the animal's needs are catabolized and excreted in the urine (Siegert et al., 2025). Accordingly, the faecal fraction is determined by the digestibility of the dietary protein, while the urinary fraction depends on how closely the supply of amino acids matches the animal's requirement (Siegert et al., 2025). NUE can therefore be improved in two main ways: by reducing the excess of protein and amino acids in the diet and by increasing the digestibility of the protein fed. A protein that is highly digestible is absorbed and made available to the animal; the part that is not digested passes through the gut and is excreted in the faeces, and is lost as faecal nitrogen (Siegert et al., 2025). Formulating diets with more digestible ingredients therefore reduces faecal nitrogen losses and raises NUE (Siegert et al., 2025). However, protein digestibility is not a fixed value, but it varies depending on a number of factors, including source (composition and structure),

processing (e.g. heat damage) and presence of antinutritional factors (e.g. trypsin inhibitors) (Calsamiglia et al., 2010).

Besides increasing digestibility, several other strategies exist to improve NUE, such as reducing the dietary CP content while balancing the essential amino acids with free amino acids (low-protein diets), precision and phase feeding, formulating diets according to the ideal protein concept, synchronizing the supply of energy and protein, genetic selection and improved manure management (Calsamiglia et al., 2010; Pomar et al., 2021; Cappelaere et al., 2021; Siegert et al., 2025).

The efficiency with which nitrogen is used differs between ruminants and monogastric animals and is generally lower in ruminants than in pigs and poultry (Pomar et al., 2021).

In dairy cattle, NUE is typically around 25%, with values ranging from about 15 to 40% depending on the diet and the animal (Lavery and Ferris, 2021). In growing dairy heifers NUE varies with age and stage, from about 15–20% in older heifers up to about 50% before weaning (Plomaritou et al., 2025). The main reason for this low efficiency lies in the rumen: part of the dietary protein is degraded to ammonia by the rumen microbes, and when ammonia exceeds the amount which microbes can use to synthetize their own protein, the surplus is absorbed, converted into urea in the liver and excreted in the urine (Calsamiglia et al., 2010; Lavery and Ferris, 2021). The amino acids that reach the small intestine come from microbial protein and from the dietary protein that escapes rumen degradation, and their digestibility in the intestine is generally around 70% (Calsamiglia et al., 2010). This intestinal digestibility depends on the protein source, on its processing and on antinutritional factors, and can therefore differ from one protein source to another (Calsamiglia et al., 2010).

In pigs, nitrogen efficiency can be improved by feeding low-protein diets balanced with free amino acids. In a study by Cappelaere et al. (2024) on growing pigs, reducing the dietary CP content raised nitrogen efficiency from about 53% to 59%. This improvement acts mainly on the urinary fraction of excreted nitrogen but has only a limited effect on the faecal fraction (Cappelaere et al., 2024). The faecal fraction, in fact, depends on the digestibility of the feed protein, and it increases when poorly digestible, fibre-rich ingredients are used (Cappelaere et al., 2021).

Poultry shows the highest nitrogen efficiency of the three categories (Pomar et al., 2021). In broiler chickens, NUE is typically around 50–65% with diets close to current commercial standards, and it can rise up to about 80–85% with low-protein diets balanced with free amino acids (Siegert et al., 2025). Because birds are uricotelic, the nitrogen from amino acids catabolised in excess is excreted mainly as uric acid (Cappelaere et al., 2021). As in pigs, the faecal nitrogen of broilers depends on the digestibility of the dietary protein: since the protein of most common ingredients is about 85–90% digestible, this undigested fraction limits the achievable NUE by roughly 10–15% (Siegert et al., 2025). Using feed ingredients with a higher protein digestibility therefore reduces this loss and increases NUE (Siegert et al., 2025).

1.3.2 Nitrogen losses and their environmental consequences

Ammonia (NH_3) is one of the main pathways of reactive nitrogen loss from livestock production (Plomaritou et al., 2025). Ammonia formation begins with the excretion of urine and faeces: the urea in the urine, which represents 50–90% of urinary nitrogen, is rapidly hydrolysed by the enzyme urease to ammonia, which is then volatilised in animal housing, during manure and

slurry storage, and after land application (Plomaritou et al., 2025; Mahmud et al., 2021). Volatilisation of ammonia is enhanced by high temperatures and by a high pH of the substrate, and it originates mainly from the urinary fraction, since faecal nitrogen is more organically bound and less volatile (Plomaritou et al., 2025; Mahmud et al., 2021). The extent of this loss is considerable: in cattle, nitrogen lost as ammonia has been estimated to account for 25-50% of manure nitrogen excretion and, in dairy cows, for about 9.3% of nitrogen intake (Hristov et al., 2011; Bougouin et al., 2016). Globally, livestock supply chains emit around 26 Tg N per year as ammonia, representing roughly 60% of total NH_3 emissions (Uwizeye et al., 2020). Because dietary protein is generally the most expensive component of diet, this volatilised nitrogen represents not only an environmental loss but also an economic one (Lavery and Ferris, 2021).

Once emitted, ammonia is highly reactive. In the atmosphere ammonia reacts with acidic species such as sulphuric and nitric acids forming secondary inorganic aerosols that contribute to fine particulate matter ($PM_{2.5}$), a recognized threat to human health associated with respiratory and cardiovascular disease (Lavery and Ferris, 2021; Mahmud et al., 2021; Wyer et al., 2022). Additionally, high ammonia concentrations within animal housing compromise the respiratory health of both animals and workers (Wyer et al., 2022). After returning to the ground through atmospheric deposition, ammonia contributes to the formation of acid rain and, when deposited on sensitive habitats, to soil acidification, eutrophication and consequent losses of biodiversity (Lavery and Ferris, 2021; Mahmud et al., 2021). In addition, part of the deposited ammonia can be re-emitted as nitrous oxide, acting as an indirect source of greenhouse gas emissions (Mahmud et al., 2021).

Part of the nitrogen excreted by animals does not leave as ammonia but remains in the soil as ammonium (NH_4^+), where it is oxidised by soil microorganisms into nitrate (NO_3^-) through the process of nitrification (Madjar et al., 2024). When the nitrogen applied to or deposited on the soil exceeds crop requirements, the excess nitrate, being highly mobile, is washed out of the soil profile and reaches both groundwater and surface waters (Madjar et al., 2024). In surface waters high nitrate concentrations promote eutrophication, while in groundwater nitrate contamination lowers the quality of water intended for human consumption and can constitute a risk to human health (Madjar et al., 2024). At a global level, livestock supply chains release around 29 Tg N per year as nitrate, representing about 39% of anthropogenic nitrate reaching surface waters and groundwater (Uwizeye et al., 2020).

Nitrous oxide (N_2O) is produced during both nitrification and denitrification (Mahmud et al., 2021). Although nitrous oxide is released in much smaller amounts than ammonia or nitrate, it is a particularly harmful compound, being a greenhouse gas with a global warming potential (GWP_{100}) about 273 times that of carbon dioxide and having an atmospheric lifetime of approximately 109 years (IPCC, 2021; Mahmud et al., 2021). Besides acting as a greenhouse gas, nitrous oxide also contributes to the depletion of the stratospheric ozone layer (Mahmud et al., 2021). Agricultural activities are the largest anthropogenic sources of atmospheric N_2O emissions, and livestock supply chains alone release about 2 Tg N per year as nitrous oxide, representing around 32% of N_2O emissions (Mahmud et al., 2021; Uwizeye et al., 2020).

Nitrogen losses through these pathways derive from the proportion of dietary nitrogen that is not retained by the animal and is consequently excreted. Faecal nitrogen excretion is primarily influenced by the digestibility of

dietary protein, which varies among protein sources (Siegert et al., 2025; Calsamiglia et al., 2010). Therefore, the selection of highly digestible protein sources represents an effective nutritional strategy to reduce nitrogen losses.

Chapter 2: PROTEIN DIGESTION IN RUMINANT AND MONOGASTRIC ANIMALS

2.1 Dietary protein and its digestion

Dietary protein is the main source of the amino acids that animals require for growth, maintenance, reproduction and synthesis of products such as meat, milk and eggs (Loveday, 2023). The protein contained in feedstuffs, however, cannot be used directly: before its amino acids can be absorbed, the protein must be hydrolysed into small peptides and free amino acids. This process, known as protein digestion, consists in the enzymatic cleavage of the peptide bonds by proteolytic enzymes under specific physicochemical conditions (Loveday, 2023; Kurz and Seifert, 2021). The efficiency of this process determines how much of the dietary protein is actually absorbed, since the undigested fraction is excreted (Kurz and Seifert, 2021).

The way in which dietary protein is digested differs profoundly between monogastric and ruminant animals. In monogastric species, such as pigs and chickens, protein is digested directly by the animal's own enzymes in the stomach and small intestine. In ruminants, by contrast, dietary protein first undergoes an extensive microbial fermentation in the forestomachs before reaching the enzymatic digestion of the small intestine.

2.2 Protein digestion in monogastric animals

2.2.1 The gastrointestinal tract and the stages of protein digestion

In monogastric species, protein digestion begins in the mouth, where feed is disrupted by chewing and mixed with saliva before being swallowed, although at this stage no proteolysis takes place (Loveday, 2023). The first

true site of protein digestion is the stomach. In the stomach, gastric juice is secreted, consisting of water, mucus, hydrochloric acid (HCl) and the enzyme pepsin (Kurz and Seifert, 2021). The combined action of the acid and of pepsin, together with the peristaltic mixing of the gastric content, breaks down the feed and starts proteolysis (Loveday, 2023). Pepsin initiates protein digestion by hydrolysing proteins into peptides and free amino acids, making them accessible for absorption in the small intestine (Kurz and Seifert, 2021). After feed intake, gastric pH temporarily increases as the ingested feed buffers gastric acid, before gradually declining again as acid secretion continues (Loveday, 2023). Proteolysis in the stomach however remains limited, serving mainly to release peptides and aromatic amino acids that signal to the gut sensing systems (Loveday, 2023). Digestion then continues in the small intestine, the main site of protein hydrolysis and of amino acid absorption. When the acidic chyme arrives into the duodenum, bile and pancreatic enzymes act on it and bicarbonate is secreted, bringing the pH close to neutral (Loveday, 2023). In this site, dietary protein is hydrolysed by pancreatic and intestinal endo- and exopeptidases into short peptides and free amino acids (Kurz and Seifert, 2021; Loveday, 2023). Bile acids and phospholipids also contribute to proteolysis by denaturing the proteins and making them more susceptible to the proteases, and the brush border membrane of enterocytes, which contains membrane-bound peptidases, complete peptide digestion prior to absorption (Loveday, 2023). A variable amount of endogenous protein — mucus, digestive enzymes and sloughed epithelial cells secreted into the gut — is added, representing a further nitrogen load (Loveday, 2023).

2.2.2 Proteolytic enzymes and physicochemical conditions

The proteolytic enzymes involved in protein digestion are divided into endopeptidases, which break peptide bonds within the protein chain, and exopeptidases, which remove amino acids from the ends of the chain (Kurz and Seifert, 2021). In the stomach, the main enzyme is pepsin. Pepsin is secreted by the gastric cells as pepsinogen, an inactive form, in order to protect the stomach from self-digestion; pepsinogen is then converted into active pepsin by the acidic environment generated by hydrochloric acid (Kurz and Seifert, 2021). Pepsin is an endopeptidase, most active at a pH of about 2, retaining partial activity up to about pH 6 (Loveday, 2023). Protein digestion then continues in the small intestine, where the proteolytic enzymes of the pancreas act. The pancreas secretes a set of proteolytic enzymes, all in an inactive form (zymogen) in order to protect the surrounding tissue from self-digestion: trypsinogen, chymotrypsinogen, proelastase and procarboxypeptidase A and B (Kurz and Seifert, 2021). The activation of these enzymes takes place in the small intestine: the enzyme enteropeptidase (enterokinase), located in the brush-border membrane of the proximal small intestine, activates trypsinogen to trypsin, and trypsin then activates the remaining zymogens in an autocatalytic cascade (Kurz and Seifert, 2021). In order to prevent premature activation within the gland, the pancreas also secretes a trypsin inhibitor (Kurz and Seifert, 2021). These enzymes hydrolyse peptide bonds in a sequence-specific manner. Among the endopeptidases, trypsin hydrolyses the peptide bonds involving the basic amino acids arginine and lysine; chymotrypsin hydrolyses the peptide bonds involving the aromatic amino acids (phenylalanine, tyrosine and tryptophan), leucine and methionine; elastase acts on neutral amino acids such as alanine, valine and glycine (Kurz and Seifert, 2021). Then the exopeptidases complete the digestion: the carboxypeptidases remove amino

acids from the carboxyl-terminal end of the peptides, while the aminopeptidases of the brush-border membrane act on the amino-terminal end (Kurz and Seifert, 2021).

The secretion of pancreatic enzymes is stimulated by feed intake and adapts, over a period of several days, to the composition of the diet; for instance, a higher dietary protein content is associated with a higher secretion of proteases such as trypsin and chymotrypsin (Kurz and Seifert, 2021). Technological processing of the feed can also influence protein digestibility, since a moderate heat treatment may improve it by inactivating enzyme inhibitors and by denaturing the proteins, making them more accessible to the proteases (Loveday, 2023).

2.2.3 Absorption of amino acids and small peptides

The final products of protein digestion — free amino acids and small di- and tri-peptides — are absorbed across the apical membrane of the enterocytes and released into the portal blood through their basolateral membrane (Kurz and Seifert, 2021; Loveday, 2023). Small peptides are taken up by a H^+-coupled symporter and are largely hydrolysed to free amino acids inside the enterocytes, although a small proportion reaches the bloodstream as intact peptides (Kurz and Seifert, 2021). Free amino acids are absorbed by different transport systems depending on their chemical nature: neutral amino acids (most of the essential amino acids) and anionic amino acids (such as glutamate and aspartate) are taken up by Na^+-coupled symporters, while cationic amino acids (such as lysine and arginine) are mostly transported by amino acid antiporters (Kurz and Seifert, 2021). Under normal conditions the protein of conventional feedstuffs is well digested in the small intestine, with a precaecal digestibility of the amino acids commonly ranging from

about 70 to 90% (Kurz and Seifert, 2021); however, a small proportion of protein and peptides can escape absorption and reach the large intestine - especially when the viscosity of the digesta is increased by dietary fibre - where the resident microbiota can break down this residual protein and use its amino acids for their own metabolism (Loveday, 2023).

2.2.4 Digesta transit and protein digestion in the large intestine

The retention time of the digesta in each segment of the gastrointestinal tract is another factor that affects digestion, since it determines how long the feed is exposed to digestive enzymes. In growing pigs fed diets containing insoluble fibre, the mean retention time of the digesta has been estimated at approximately 1 hour in the stomach, 4 hours in the small intestine and 38 hours in the large intestine (Wilfart et al., 2007). The transit is therefore relatively rapid in the compartments where enzymatic protein digestion and amino acid absorption actually take place (the stomach and the small intestine) and much slower in the large intestine, where the solid phase of the digesta moves 4 to 8 hours more slowly than the liquid phase (Wilfart et al., 2007).

The microbial fermentation of the protein that reaches the large intestine, favoured by the very long retention time in this segment, has generally been considered to have minimal nutritional value for the pig (Shurson and Kerr, 2023). In fact, the colonocytes have only a limited capacity to absorb amino acids, the microbial enzymes involved in protein degradation are present at very low levels along the porcine gastrointestinal tract, and the infusion of amino acids directly into the large intestine has shown limited or no benefit for the animal (Loveday, 2023; Kurz and Seifert, 2021; Shurson and Kerr, 2023). The nitrogen absorbed from this segment is moreover mostly in the

form of ammonia produced by the microbial fermentation of the undigested protein, and a large proportion of it is detoxified to urea in the liver and excreted in the urine, so that the fermentation of undigested protein in the large intestine is generally regarded as a loss of nitrogen rather than as a contribution to the protein nutrition of the animal (Shurson and Kerr, 2023). More recent evidence, however, partly contradicts this view: part of the urea is recycled into the gastrointestinal tract and once returned to the small intestine, is hydrolysed to provide nitrogen for microbial amino acid synthesis that can be absorbed and used by the animal (Shurson and Kerr, 2023). Additionally, in one study about 80% of the nitrogen infused into the large intestine was absorbed and whole-body nitrogen retention improved, which suggests that nitrogen metabolism in the large intestine should not be entirely neglected (Shurson and Kerr, 2023).

2.2.5 Protein digestion in poultry

In the chicken the foregut is organised very differently from that of the pig. It comprises the beak, the esophagus, the crop and a two-part stomach, which is split into a glandular chamber (the proventriculus) and a muscular chamber (the gizzard) instead of the single stomach of mammals (Rodrigues & Choct, 2018). Food is collected by the beak and swallowed whole with limited saliva production (Rodrigues & Choct, 2018). Salivary glands are present (daily secretion has been reported to range from about 7 to 25 mL), but their secretion only lubricates the feed and helps it pass through the esophagus (Rodrigues & Choct, 2018). No relevant enzymatic protein digestion therefore takes place in the mouth. When feed is not continuously available, it first reaches the crop, a ventral sac-like dilatation of the esophagus that can distend and store feed particles thanks to the longitudinal folds on its inner

surface (Rodrigues & Choct, 2018). By contrast, under continuous (ad libitum) feeding, chickens eat small and frequent meals and feed largely bypasses the crop (Svihus, 2014). The crop does not secrete digestive enzymes and does not absorb nutrients, so its only role is to store and moisten the feed (Rodrigues & Choct, 2018). This storage function is necessary because the gizzard can hold no more than 5 to 10 g of feed, so that large meals must be buffered in the crop before being released gradually to the stomach (Svihus, 2014). Whether the feed actually enters the crop depends on the state of the stomach: when the gizzard is full, the entrance of the crop opens and the feed is stored there; when the gizzard is empty, the feed passes directly to the stomach (Rodrigues & Choct, 2018; Svihus, 2014). Inside the crop, feed is progressively moistened, reaching about 50% moisture within roughly 60 to 90 minutes of retention (Svihus, 2014; Rodrigues & Choct, 2018). This softening is not a digestive step, but by hydrating the feed it can make the subsequent grinding in the gizzard and the enzymatic breakdown in the small intestine more effective (Svihus, 2014). The pH of the crop is variable and generally mildly acidic to near neutral, with reported values ranging from about 3.8 to 6.9 depending on the feed and on the retention and fermentation time (Rodrigues & Choct, 2018). From the crop the feed moves to the proventriculus, or glandular stomach, the functional counterpart of the mammalian stomach (Rodrigues & Choct, 2018). The deep glands of the proventriculus release hydrochloric acid and pepsinogen into the lumen, starting the chemical digestion of protein (Rodrigues & Choct, 2018). As in the pig, the acidic environment converts pepsinogen into active pepsin, which starts to hydrolyse the dietary protein into shorter peptides. However, because the proventriculus has a small volume, the feed usually flows rapidly through it, and most of the digestive action of these secretions actually takes

place in the gizzard, where acid, enzymes and feed are mixed by the muscular contractions (Rodrigues & Choct, 2018; Svihus, 2014).

The gizzard, or muscular stomach, is a strong muscular organ whose thick walls physically break down and grind the feed; in birds this grinding replaces the chewing that does not occur in the mouth (Rodrigues & Choct, 2018; Svihus, 2014). Its inner surface is covered by a hard carbohydrate–protein layer, the koilin, which protects the wall from the acid and proteolytic enzymes arriving from the proventriculus and from the abrasion caused by grinding hard particles (Rodrigues & Choct, 2018). Feed particles are reduced by grinding against the koilin and against one another until they become small enough (generally under about 0.1 mm) to leave the gizzard and pass into the duodenum (Rodrigues & Choct, 2018). Because of the repeated contractions, material is refluxed back and forth between the gizzard and the proventriculus several times; for this reason, the gizzard and the proventriculus are best considered as a single functional compartment, in which the feed is repeatedly re-exposed to acid and pepsin while being ground (Svihus, 2014). The pH of the gizzard content is acidic but variable: values between about 1.6 and 4.5 have been reported, and it is generally around 3 to 4 in birds fed standard pelleted diets (Rodrigues & Choct, 2018; Svihus, 2014). This pH range is well suited to pepsin activity, whose optimum has been reported at about 2.8 (Rodrigues & Choct, 2018).

The passage of feed through the chicken foregut is rapid: it spends about 10 to 50 minutes in the crop (depending on feed availability and lighting regime) and about 30 to 90 minutes in the proventriculus-gizzard (depending on feed structure and form), for a total of no more than 3 to 4 hours (Rodrigues & Choct, 2018).

In the small intestine the process is essentially the same as in the pig: in this site most of the dietary protein is digested and almost all of the amino acids are absorbed (Svihus, 2014). When the acidic content leaves the gizzard, it is mixed with bile and pancreatic secretions through gastroduodenal refluxes during the very short retention in the duodenum, so that the pH rises above 6 and enzymatic digestion begins (Svihus, 2014). Dietary protein is then hydrolysed by the pancreatic and brush-border enzymes into peptides and free amino acids that are absorbed across the enterocytes, as described for the monogastric model. Digestion and absorption of protein are largely completed by the end of the jejunum, while the ileum is mainly involved in the absorption of water and minerals (Svihus, 2014).

2.2.6 Measurement of protein digestibility: ileal digestibility

In monogastric animals the digestibility of dietary protein is commonly expressed as the ileal digestibility of amino acids, measured at the end of the small intestine (the terminal ileum) rather than in the faeces. Because the amino acids that escape absorption in the small intestine are fermented by the microbial population of the hindgut, which changes their composition, faecal values do not reflect the amino acids actually available to the animal; ileal digestibility is therefore preferred as the more accurate measure (Kong and Adeola, 2014; Loveday, 2023). In the ileum, the amino acids in the digesta are not only of dietary origin but also include the ileal endogenous amino acid losses (IAAend) - proteins secreted by the animal itself, such as digestive enzymes, mucus and sloughed epithelial cells. These endogenous losses can be separated into basal losses, which do not depend on the feed ingredient, and specific losses, which are induced by ingredient characteristics such as antinutritional factors and dietary fibre (Stein et al.,

2007). Depending on how these endogenous losses are taken into account, ileal digestibility can be expressed in three ways: the apparent ileal digestibility (AID) is obtained by subtracting the total amino acid outflow at the ileum from the amount ingested, without correcting for endogenous losses; the true ileal digestibility (TID) corrects the apparent value for the total endogenous losses, so that it reflects only the undigested dietary amino acids; and the standardized ileal digestibility (SID) corrects only for the basal endogenous losses (Stein et al., 2007; Kong and Adeola, 2014). The standardized ileal digestibility is the measure recommended for the formulation of swine and poultry diets, as it restores the additivity of the values, allowing the digestibility of a complete diet to be predicted by summing the contributions of the individual ingredients (Kong and Adeola, 2014; Stein et al., 2007).

2.3 Protein digestion in ruminants

2.3.1 The rumen microbial ecosystem

The digestion of dietary protein in ruminants differs fundamentally from that of monogastric animals, as it is preceded by an extensive microbial fermentation in the rumen (Bach et al., 2005). The rumen is a large fermentation chamber inhabited by a dense and diverse microbial community, which carries out the first stage of protein digestion (Qi et al., 2024; Bach et al., 2005). This compartment is a strictly anaerobic environment, with a pH generally maintained between 6 and 7 (Matthews et al., 2019).

The rumen microbiota is the product of a long coevolution between the micro-organisms and their host: the host offers a stable environment and a steady supply of fermentable substrates, and in return the microbes break

down plant constituents such as cellulose, hemicellulose and starch, yielding energy and nutrients for the animal (Qi et al., 2024). Bacteria are the most numerous micro-organisms in the rumen, reaching 10^{10} to 10^{11} cells/mL and comprising more than 200 species (Matthews et al., 2019); they are also the functionally most diverse group, and include cellulolytic, amylolytic, proteolytic, peptidolytic and deaminating species (Qi et al., 2024). Protozoa, anaerobic fungi and methanogenic archaea are present in smaller quantity: ciliate protozoa at 10^4 to 10^6 cells/mL, anaerobic fungi at 10^3 to 10^6 zoospores/mL (about 8 to 12% of the total microbial biomass), and methanogenic archaea at 10^6 to 10^8 cells/mL, the last representing less than 4% of the microbial community (Matthews et al., 2019).

Because the feed first encounters this microbial population, dietary protein is degraded by the rumen microbes rather than by the animal's own enzymes, and part of the resulting compounds is used to build microbial protein (Bach et al., 2005). What then leaves the rumen and reaches the small intestine is therefore a mixture of microbial protein and of dietary protein that has escaped ruminal degradation (Bach et al., 2005).

2.3.2 Ruminal degradation of dietary protein (RDP)

Based on its fate in the rumen, the crude protein (CP) of the feed is divided into two fractions: rumen-degradable protein (RDP), which is degraded by the micro-organisms in the rumen, and rumen-undegradable protein (RUP), which escapes degradation and reaches the small intestine (NASEM, 2021). The RDP includes both the non-protein nitrogen (NPN) and the fraction of true protein that is degraded in the rumen (Bach et al., 2005). The proportion of a protein that ends up as RDP or RUP is not a fixed property of the feed

but depends on the balance between its rate of degradation and its rate of passage through the rumen (NASEM, 2021).

The degradation of dietary protein in the rumen begins with the attachment of bacteria to feed particles: about 70 to 80% of the ruminal micro-organisms are attached to the feed, and approximately 30 to 50% of these show proteolytic activity (Bach et al., 2005). In the rumen, a community of different species acts together, and their proteases hydrolyse the dietary protein into peptides and free amino acids (Bach et al., 2005). Bacteria carry out most of this proteolysis, although ciliate protozoa account for about 20% of the ruminal proteolytic activity, and some anaerobic fungi also exhibit proteolytic capacity (Qi et al., 2024). The peptides and amino acids released by proteolysis are taken up by the microbial cells, where the peptides are further hydrolysed to amino acids by microbial peptidases (Bach et al., 2005). Depending on energy availability, these amino acids follow different metabolic fates. When fermentable energy (carbohydrate) is available, these amino acids are used directly for the synthesis of microbial protein, whereas when energy is limiting they are deaminated: their carbon skeletons are fermented to volatile fatty acids and their nitrogen is released as ammonia (Bach et al., 2005; Qi et al., 2024). Ammonia is therefore the central intermediate of nitrogen metabolism in the rumen, and it is the main nitrogen source used by many rumen bacteria to build their own microbial protein (Bach et al., 2005). When the rate of protein degradation and deamination exceeds the rate at which microbes can capture ammonia into microbial protein, however, ammonia accumulates in the rumen. Because ammonia is easily absorbed across the rumen wall, the excess passes into the blood, is converted to urea in the liver, and is then partly recycled to the gut and partly excreted in the urine (Calsamiglia et al., 2010). This imbalance between the production of ammonia and its capture into microbial protein is one of the

main reasons why the efficiency of nitrogen utilisation in ruminants is typically low, around 25%, and highly variable, from 10 to 40% (Calsamiglia et al., 2010).

The ruminal degradability of a dietary protein depends largely on its own characteristics, in particular its solubility and structure. Generally, soluble proteins such as globulins are rapidly and extensively degraded, while insoluble proteins such as prolamins and glutelins are degraded more slowly (Bach et al., 2005). Protein structure, however, also plays a role, since disulphide bonds can make a protein resistant even when it is soluble: the glycinin of soybean, for instance, is a globulin stabilised by strong disulphide bonds and is therefore fairly resistant to ruminal degradation (Bach et al., 2005). Protein degradation also depends on the time the feed spends in the rumen, as it is inversely related to the passage rate; a rapid passage through the rumen, indeed, leaves the microbes less time to degrade the protein (Bach et al., 2005).

2.3.3 Microbial protein synthesis

The rumen micro-organisms use the nitrogen released by protein degradation (mainly as ammonia, but also as peptides and amino acids), together with the energy obtained from carbohydrate fermentation, to synthesise their own protein, known as microbial protein (Bach et al., 2005). Microbial protein is of central importance for the ruminant, since it provides the majority of protein supplied to the small intestine, representing roughly 50 to 80% of total absorbable protein (Bach et al., 2005; NASEM, 2021). About 80% of the microbial crude protein is true protein, with a relatively constant amino acid profile, which makes it a protein of good nutritional value for the animal (NASEM, 2021). The amount of microbial protein that enters the small

intestine depends on the availability of nutrients, in particular of fermentable energy and nitrogen, and on the efficiency of microbial protein synthesis (Bach et al., 2005). This synthesis, however, is never fully efficient, because the micro-organisms use only part of their energy for growth and spend the rest on maintenance, on the storage of reserve carbohydrate, and on "energy spilling", futile metabolic cycles that dissipate energy as heat (Hackmann and Firkins, 2015).

2.3.4 Rumen-undegradable protein (RUP), metabolizable protein (MP) and post-ruminal digestion

The rumen-undegradable protein (RUP) is the fraction of dietary protein that escapes microbial degradation in the rumen and passes to the small intestine, where it is digested by the animal's own enzymes as in monogastric animals (NASEM, 2021). Together with the microbial protein synthesised in the rumen, the digestible RUP makes up the metabolizable protein (MP), defined by NASEM as the true protein digested postruminally, which represents the protein actually available to the ruminant for absorption (NASEM, 2021). Only the fractions of the microbial protein and of the RUP that are digested to amino acids and absorbed in the small intestine actually contribute to the metabolizable protein (Owens et al., 2014).

The protein that leaves the rumen, made up of microbial protein, RUP and endogenous protein, is then digested in the abomasum and the small intestine. The abomasum is the true glandular stomach, functionally equivalent to the stomach of monogastric animals, and from this point the enzymatic digestion of protein follows the same steps as in the monogastric model, with acid and pepsin in the stomach, pancreatic and brush-border enzymes in the small intestine, and the absorption of the resulting amino

acids and small peptides. The intestinal digestibility of this protein, however, is not the same for all its fractions. Microbial true protein is highly digestible, and its digestibility in the small intestine is assumed to be about 80% (NASEM, 2021). The intestinal digestibility of the RUP, instead, varies considerably among feeds, as it depends on the protein source and on the treatment applied to the feed; an excessive heat treatment, for instance, damages the protein by cross-linking its amino acids, thereby lowering its intestinal digestibility (NASEM, 2021).

2.3.5 Measurement of protein digestibility: ruminal degradation and intestinal digestibility

Protein digestibility in ruminants is evaluated considering both the ruminal degradation of dietary protein and the intestinal digestibility of RUP. Ruminal protein degradability is commonly measured in situ by incubating feed samples in nylon bags placed in the rumen of cannulated animals and measuring the disappearance of protein over increasing incubation times (Harstad and Prestløkken, 2001; Wang et al., 2015). The intestinal digestibility of RUP can be then determined using the mobile nylon bag technique or in vitro procedures (Wang et al., 2015).

Chapter 3: CONVENTIONAL OILSEEDS AND ANIMAL PROTEIN SOURCES

The protein sources evaluated in this study and compared to black soldier fly larvae meal were selected to represent classical feedstuffs used in animal nutrition: two oilseeds, soybean and sunflower, and one animal-derived protein authorised in the European Union, herring meal. The oilseeds were used as whole, full-fat seeds rather than as defatted meals obtained after oil extraction, in order to provide a lipid content as close as possible to that of insect meal.

3.1 Herring Meal

3.1.1 Origin and production

Herring meal is a type of fish meal, a protein-rich feed ingredient obtained from whole fish or from the by-products of fish processing (Heuzé et al., 2026). Most of it is produced from small, oily and bony fish that have little value for direct human consumption, or from the by-products of the processing of fish destined for human food (Cho et al., 2011).

Fish meal is obtained mainly from small pelagic species caught for this purpose, such as anchovy, menhaden, sardine, capelin and herring (Heuzé et al., 2026). The fish used to produce fish meal are generally divided into two groups. Lean fish, such as cod and haddock, are valued for their fillets, so their meal is mainly made from the residues left after filleting. Oily fish, by contrast, are not used for fillets and are known as industrial fish. Herring belongs to this second group, and about 90% of the world fish meal production comes from these high-oil species (Miles and Jacob, 2011).

The production process involves several steps. Cooking first coagulates the proteins and sterilises the material, preparing it for the removal of a liquid fraction that contains oil, water and protein. This liquid is then separated by pressing from the solid residue, called press cake, and the oil is recovered from the liquid by centrifugation. The remaining watery fraction, known as stick water, is rich in soluble protein and is concentrated and added back to the press cake, which is finally dried and ground to give the meal (Miles and Jacob, 2011).

3.1.2 Nutritional composition and protein digestibility

As a fish meal, herring meal is an ingredient of high nutritional value, characterized by a high protein content and a complete amino acid profile (Cho et al., 2011). The CP content of fish meal generally ranges between 60 and 72% (Cho et al., 2011); on an as-fed basis, a typical high-protein fish meal contains roughly 69% crude protein, 9% fat and 14% ash (INRAE-CIRAD-AFZ, 2024). The quality of its protein depends on its amino acid profile, since, unlike most plant proteins, fish meal provides a complete and well-balanced profile, particularly rich in the amino acids that are often limiting in animal diets, such as lysine and methionine (Cho et al., 2011). In a 70% fish meal, lysine and methionine reach about 52 and 19 g per kilogram, respectively (INRAE-CIRAD-AFZ, 2024). Fish meal is also a natural source of long-chain n-3 (omega-3) polyunsaturated fatty acids, especially EPA and DHA, and of trace minerals such as selenium and iodine (Cho et al., 2011).

In monogastric species, fish meal protein is highly digestible. In growing pigs, the standardised ileal digestibility (SID) of crude protein in fish meal reaches 83.4%, and the SID of lysine, the first-limiting amino acid, is 85.4%

(Kim et al., 2022). A comparable pattern is found in broiler chickens, where the ileal digestibility of the essential amino acids in fish meal ranges from about 78 to 86%, with lysine and methionine both digested at 86% (Lemme et al., 2004). In ruminants, herring meal behaves as a high-quality bypass protein. When incubated in the rumen, only about 25% of its crude protein is degraded after 16 hours (a time that corresponds to a typical rumen retention), so that a large fraction escapes ruminal fermentation and reaches the small intestine as rumen-undegraded protein (RUP) (Harstad and Prestløkken, 2001). This undegraded protein is then almost completely digested, since its true intestinal indigestibility is only 1.4%, which corresponds to an intestinal digestibility of about 98.6% (Harstad and Prestløkken, 2001).

3.1.3 Use in animal feeding

Fish meal is valued in animal nutrition as a concentrated, high-quality protein source, but the amount that can be produced depends on catches of wild fish, so that its availability is limited and its price tends to be high and variable (Heuzé et al., 2026). On the Italian market, high-protein fish meal (70 to 72% CP) was quoted at about €3,202 to €3,227 per tonne in July 2026, about six times the price of roasted full-fat soybean (Borsa Merci di Bologna, 2026). Most of the global production of fish meal is directed to aquaculture, so only a smaller part is available for terrestrial livestock (Heuzé et al., 2026). In pigs, fish meal is included mainly in the diets of weaned piglets, because this category is particularly sensitive to the anti-nutritional factors of soybean meal (such as glycinin and β-conglycinin) and because fish meal supplies well-balanced amino acids during this delicate phase (Kim et al., 2022). In poultry, fish meal is one of the animal protein sources commonly used in

broiler nutrition, where it is valued for its high content of essential amino acids such as methionine and lysine (Lemme et al., 2004; Jacob, 2020). Its inclusion in poultry diets is usually limited to about 5 to 10% (Jacob, 2020). In ruminants, although fish meal is an excellent source of RUP, its use is prohibited in the European Union under the feed ban introduced after the BSE crisis, and it is authorised only for non-ruminants and for young ruminants fed on milk replacers in dry form (Regulation (EC) No 999/2001; Regulation (EC) No 956/2008).

3.1.4 Sustainability and competition with human food

An important sustainability concern related to fish meal comes from its origins, since much of it is obtained from wild-caught fish, mainly small pelagic species such as anchovies and sardines, that are caught specifically to be reduced into fish meal and fish oil, an activity known as reduction fishery (Majluf et al., 2024). Although these small pelagic fish have traditionally been considered of little value for direct human consumption, they are edible and nutritious, and their use as feed raises the issue of competition with human food (Majluf et al., 2024). This concern is especially serious because reduction fisheries often operate in areas where low-income coastal communities depend on these same fish for food and income, so using the catch for feed production can affect their food security and livelihoods (Majluf et al., 2024). An example of this conflict between direct human consumption and reduction to feed is the case of Atlantic herring in the Baltic Sea, where the pelagic sector is dominated by herring and sprat, and where the share of landings destined for reduction to fish meal and oil has risen from about 50% in 2013 to around 77% by 2022 (Hornborg, 2023). Beyond the competition with human food, the production of fish meal from wild fish also raises environmental concerns. Reduction fisheries compete

with marine predators, such as seabirds and marine mammals, for the same small pelagic fish, possibly affecting the productivity and resilience of marine ecosystems (Majluf et al., 2024). These concerns apply especially to fish meal produced from whole fish. When fish meal is instead made from the by-products of fish processing, such as heads, frames and trimmings, it valorises material that would otherwise be discarded, and it therefore improves the use of marine resources (Heuzé et al., 2026). The share of fish meal produced from waste and by-products is expected to increase to about 31% by 2034, so most of it will still come from whole fish (Heuzé et al., 2026).

3.2 Roasted full-fat soybean

3.2.1 Origin and heat treatment

Soybean (*Glycine max*) is the largest oilseed crop in the world, mainly produced in the United States, Brazil and Argentina (Heuzé et al., 2017). From the seed, two main products are used in animal feeding: soybean meal, the defatted fraction that remains after the oil has been extracted, and full-fat soybean, the whole seed that still contains its oil and therefore provides more energy than the meal (Heuzé et al., 2017; Stein et al., 2008). Raw soybeans cannot be used directly in animal feeding because they contain antinutritional factors, particularly trypsin inhibitors (Heuzé et al., 2017; Hoffmann et al., 2003). For this reason, before being fed, the seeds are subjected to heat treatment to denature inhibitor proteins (Hoffmann et al., 2003). The whole soybean seed after a heat treatment is called roasted full-fat soybean. Monogastric animals are more sensitive to these antinutritional factors than ruminants, because in ruminants trypsin inhibitors are inactivated and

degraded by microbial fermentation in the rumen, whereas monogastric species do not have this protection (Hoffmann et al., 2003). Thus, while in monogastric diets, especially in those of young pigs and poultry, the heat treatment serves to inactivate antinutritional factors, in ruminant diets it is used to increase the proportion of protein that escapes ruminal degradation (Heuzé et al., 2017). Toasting and extrusion are among the most common of these treatments (Heuzé et al., 2017). The intensity of the heating must be carefully controlled, as insufficient heating leaves the antinutritional factors active, while excessive heating can reduce protein digestibility (Heuzé et al., 2017).

3.2.2 Nutritional composition and protein digestibility

Among the seeds commonly used in animal feeding, soybean is the richest in protein (Heuzé et al., 2017). On an as-fed basis, roasted full-fat soybean contains about 35.8% CP, 19.7% fat and 4.9% ash, with a low content of starch (5.1%) and a moderate amount of fibre (NDF 12.2%, ADF 7.2%) (INRAE-CIRAD-AFZ, 2024). Soybean protein is rich in lysine but relatively poor in the sulphur amino acids, methionine and cysteine, which are its first-limiting amino acids (Stein et al., 2008). In roasted full-fat soybean, lysine reaches about 22.2 g per kilogram and methionine about 5.2 g per kilogram (INRAE-CIRAD-AFZ, 2024). Because cereal grains show the opposite pattern, being poor in lysine, soybean and cereals complement each other well in animal diets (Stein et al., 2008).

In pigs, the standardised ileal digestibility of the crude protein of roasted full-fat soybean is about 74% (INRAE-CIRAD-AFZ, 2024). The effect of heat treatment is clearly shown in broilers, where the apparent ileal digestibility of crude protein was 76.8% for a diet containing properly deactivated full-

fat soybean, but fell to 65.3% when the soybean was raw and to 65.7% when part of it had been over-heated (Rocha et al., 2014). The lower value observed when the soybean had been over-heated is explained by the damage that excessive heating causes to heat-sensitive amino acids such as lysine, methionine and cystine, reducing their digestibility (Rocha et al., 2014). According to Stein et al. (2008), lysine level can also decrease due to total destruction during the formation of advanced Maillard reaction products. In ruminants, the aim of the heat treatment is to reduce the ruminal degradation of the soybean protein, so that a larger fraction escapes the rumen as RUP and reaches the small intestine (Heuzé et al., 2017). In roasted full-fat soybean, this rumen-undegraded protein represents about 35 to 42% of the crude protein and it is then digested in the small intestine, with an intestinal digestibility of about 82% (INRAE-CIRAD-AFZ, 2024). The degree of heating must be balanced: as heat input increases, a larger fraction of the protein escapes ruminal degradation, but at the same time the availability of lysine falls, because heat makes it unavailable through the Maillard reaction (Faldet et al., 1992). The treatment that maximises the amount of available lysine reaching the small intestine requires a loss of about 15 to 22% of the available lysine of the seed, and it increases this supply by about 56% compared with raw soybeans (Faldet et al., 1992).

3.2.3 Use in animal feeding

Full-fat soybean is used in animal feeding as a source of both protein and energy, and it is valued especially in the diets of animals with high energy requirements, since, in addition to its protein, it supplies the energy of its oil (Stern and Illg, 1988). In pigs, full-fat soybean is an excellent protein source, used especially in the diets of weaned and growing pigs, although its

relatively high price limits its use (Wang et al., 2023); on the Italian market it was quoted at about €514 to €517 per tonne in July 2026 (Borsa Merci di Bologna, 2026). In broilers, roasted or extruded full-fat soybean is also an excellent protein source, usually included at up to about 25% of the diet (Stein et al., 2008). In ruminants, full-fat soybean is used in cattle and sheep as a source of both high-quality protein and energy, the latter coming from its high fat content, which is useful for high-producing animals such as dairy cows; however, an excessive intake of unprocessed soybean fat can depress fibre digestion in the rumen, so its inclusion must be limited (Stern and Illg, 1988).

3.2.4 Sustainability and competition with human food

Soybean meal is the main protein source used in the livestock and poultry industries worldwide, and soybean is the largest source of vegetable protein for animals globally (Stein et al., 2008; Rotundo et al., 2024). Most of the soy produced, about 75% by weight, is used as feed for livestock rather than eaten directly by people, even though soybean is also an important human food (Fraanje and Garnett, 2020; Heuzé et al., 2017). The large scale of soybean production has an important environmental cost: soybean cultivation is a major driver of land-use change and deforestation in the Amazon and other regions of South America (Fraanje and Garnett, 2020). The three largest producers are the United States, Brazil and Argentina, which together account for about 82% of world production; while the United States has been a major producer for decades, it is the recent and enormous expansion of soy in South America that is most closely linked to this deforestation (Fraanje and Garnett, 2020). Because of this link to deforestation, soybean has a large carbon footprint, and its cultivation is

associated with negative impacts on biodiversity (Rotundo et al., 2024). Europe is particularly dependent on imported soy: the EU-27 and the United Kingdom import about 33 million tonnes of soybean products every year, predominantly for livestock feed and mainly from Brazil, the United States and Argentina (Rotundo et al., 2024).

On the other hand, soybean is a nitrogen-fixing legume, so it is usually grown with little or no nitrogen fertiliser and can improve the soil when grown in rotation with other crops (Heuzé et al., 2017; Rotundo et al., 2024). It has been estimated that increasing soybean production within Europe could reduce greenhouse gas emissions by 37 to 291 million tonnes of CO_2 equivalent per year and nitrogen fertiliser use by 0.6 to 1.2 million tonnes per year (Rotundo et al., 2024).

Because of these environmental concerns, the partial replacement of soybean in livestock feeds with more sustainable alternatives has been proposed as a way to reduce the environmental impact of animal production (Pexas et al., 2023).

3.3 Whole sunflower seed

3.3.1 Sunflower seed as a feed

Sunflower (*Helianthus annuus*) is an oilseed crop native to North America, today cultivated worldwide (Jacob, 2014). Sunflower seeds are characterised by a high oil content, from about 40 to 55%, together with a moderate amount of protein, and are rich in unsaturated fatty acids, in particular linoleic acid (Rodríguez et al., 1998; Jacob, 2014). The composition of the seed depends on the variety and on the growing conditions, which is one of the reasons why the values reported in the literature vary (Jacob, 2014). Two types of

sunflower are grown: the high-oil type, which contains from 40 to 51% oil and is the one mainly used to produce sunflower oil, and the confectionery type, which has a lower oil content (around 25%) and is used mostly for snacks and bird food (Jacob, 2014). When the seed is processed for its oil, the residue that remains after the oil has been extracted is called sunflower meal and is used as a protein feed, whereas the whole seed keeps all of its oil, providing much more energy (Jacob, 2014; Selvaraj and Purushothaman, 2004).

Unlike the other major oilseeds such as soybean, cottonseed and rapeseed, whole sunflower seeds do not have antinutritional factors, and for this reason they are considered to be a safe feed ingredient for all species (Morsy et al., 2015). Because they do not contain these factors, whole sunflower seeds do not require the heat treatment that raw soybean needs to inactivate its trypsin inhibitors. The seeds contain phenolic compounds, mainly chlorogenic acid, which can react with proteins and were therefore suspected of reducing protein digestibility; in studies conducted in broilers on sunflower seed, however, this effect was not observed, and neither the protein nor the amino acids showed a lower digestibility (Rodríguez et al., 1998). The main limitation of the whole seed is instead its hull, which is rich in fibre. This fibre is strongly lignified, making the fibre fraction poorly digestible (Selvaraj and Purushothaman, 2004).

3.3.2 Nutritional composition and protein digestibility

The most abundant component in whole sunflower seed is the oil, which represents from about 38 to 48% of the dry matter, while the crude protein content is moderate, from about 16 to 22% (INRAE-CIRAD-AFZ, 2024; Selvaraj and Purushothaman, 2004; Rodríguez et al., 1998; Morsy et al.,

2015). Whole sunflower seed is rich in fibre, most of which comes from the strongly lignified hull, with a lignin content of about 6%, and it contains very little starch (around 1%) and about 3% of ash (INRAE-CIRAD-AFZ, 2024). Compared with soybean, the protein of sunflower is poorer in lysine but richer in the sulphur amino acids methionine and cystine. In the whole sunflower seed lysine represents about 4.1 g per 16 g of nitrogen and the sum of methionine and cystine about 4 g per 16 g of nitrogen, whereas in roasted full-fat soybean lysine is higher, about 6.2 g, and the sum of methionine and cystine lower, about 2.9 g per 16 g of nitrogen (INRAE-CIRAD-AFZ, 2024). The digestibility of the protein of whole sunflower seed has been studied in pigs, poultry and ruminants. In pigs, the standardised ileal digestibility (SID) of crude protein is about 82% (INRAE-CIRAD-AFZ, 2024). The digestibility of lysine is lower than that of the other amino acids (about 75%), so lysine is not only scarce in sunflower but also less available (INRAE-CIRAD-AFZ, 2024). In poultry, the apparent digestibility of crude protein of the seed, measured over the whole tract in adult roosters fed the seed alone, was about 80% (Selvaraj and Purushothaman, 2004). In ruminants, the protein of whole sunflower seed is highly degraded in the rumen, with a rumen degradability of about 82%, so only a small part of the protein escapes to the small intestine (INRAE-CIRAD-AFZ, 2024). This rumen-undegraded fraction is then digested in the intestine at about 80%, and the overall apparent digestibility of the nitrogen over the whole tract is about 67% (INRAE-CIRAD-AFZ, 2024). Because whole sunflower seed is fed raw, most of its protein is degraded in the rumen, differently from the roasted full-fat soybean, which is heat-treated precisely to increase the proportion of protein that escapes ruminal degradation, up to about 35 to 42% (INRAE-CIRAD-AFZ, 2024).

3.3.3 Use in animal feeding

Because of its high oil content, whole sunflower seed is used in animal feeding mainly as a source of energy, although it also provides a moderate amount of protein (Heuzé et al., 2015). Unlike soybean, it does not need any heat treatment, and it is a cheaper energy source than other oilseeds (Heuzé et al., 2015). Its inclusion in the diet is limited mainly by two characteristics: the fibre of the hull and the high content of unsaturated oil, whose importance differs from one species to another (Heuzé et al., 2015).

In ruminants the seed is palatable and can be fed raw, with no need for processing, because animals break it down during rumination (Heuzé et al., 2015). In these animals whole sunflower seed is used as a source of energy and, thanks to its oil rich in unsaturated fatty acids, to improve the fatty acid profile of milk and meat (Heuzé et al., 2015). Since the seed is high in fat, large amounts can depress feed intake and milk production, so the inclusion is kept moderate, around 7.5 to 10% of the diet in dairy cows and up to about 15% in finishing beef cattle (Heuzé et al., 2015; McGuffey and Schingoethe, 1982). In pigs, sunflower seed is valued for its energy, but its fibre content limits its use in growing animals, and the recommended inclusion is about 8 to 10% for growing and finishing pigs and up to 25% for sows (Heuzé et al., 2015). In a digestibility trial in pigs conducted by Kepler et al. (1981), ground sunflower seed was added to the diet at 0%, 25% and 50%; including it at 25% did not reduce the digestibility of the diet compared with the control, while at 50% the digestibility of some fractions, such as dry matter and fibre, decreased. The digestibility of the protein stayed stable at all the levels tested (Kepler et al., 1981). The main constraint in pigs is related to fat quality, since the highly unsaturated oil of the seed can make the body fat

too soft, so the amount of linoleic acid in the diet has to be limited (Heuzé et al., 2015). In poultry whole sunflower seed is a high-energy feed that can be fed raw, and it is commonly included at up to 25 to 30% of the diet (Heuzé et al., 2015). In broilers, the inclusion of the seed up to 25% of the diet did not reduce weight gain or feed efficiency (Rodríguez et al., 1998). In another trial, the inclusion up to 20% did not affect performance and even improved the feed conversion ratio (Selvaraj and Purushothaman, 2004). The high fat content also increases fat deposition, particularly abdominal fat in broilers (Heuzé et al., 2015; Selvaraj and Purushothaman, 2004)

3.3.4 Sustainability and competition with human food

Primarily grown for oil extraction, sunflower is the fourth most important oilseed crop worldwide (Heuzé et al., 2015; Adeleke and Babalola, 2020). Its oil is considered a high-quality edible oil and is extensively utilized for human consumption (Adeleke and Babalola, 2020). Vegetable oils are among the feed materials that compete with human food, together with cereals, whole fish and pulses, and replacing this group of food-competing feedstuffs with food system by-products and residues could increase the global food supply by up to 13% in terms of energy and 15% in terms of protein (Sandström et al., 2022). Since whole sunflower seed still contains its oil, which has a direct human food use, feeding the whole seed competes with human food more than feeding the sunflower meal that remains after oil extraction, which is instead a by-product.

Sunflower crop is often considered as environmental-friendly and agroecological, since it requires limited amounts of nitrogen fertiliser, no irrigation and a limited use of pesticides (Debaeke et al., 2017). It is also a

rustic crop, tolerant to water stress, that can be grown in low-input systems and used to diversify crop rotations (Debaeke et al., 2017).

Chapter 4: *HERMETIA ILLUCENS* AS AN ALTERNATIVE PROTEIN SOURCE

4.1 Insects as feed

Since protein is the most costly and limiting ingredient in animal diets, and the growing demand for animal products is making its supply increasingly challenging, there is a strong interest in finding alternative protein sources (Lu et al., 2022). Among these alternatives, insects are regarded as one of the most promising protein sources for animal feeding (Shah et al., 2022). Insects offer several advantages as a feed. Firstly, they have a high protein content and a high protein quality in terms of essential amino acids (Shah et al., 2022). Secondly, their short life cycle allows them to be reared on a large scale and at competitive prices compared with the other species proposed as animal feed (Shah et al., 2022). Additionally, many insect species can be reared on organic by-products and wastes, turning low-value material into protein-rich edible biomass and closing nutrient loops (Wang and Shelomi, 2017). The insects used as feed for monogastric animals include the black soldier fly, grasshoppers, mealworms, housefly larvae and crickets (Shah et al., 2022). Among them, the black soldier fly (*Hermetia illucens*) is considered one of the most promising insects for animal feed (Shah et al., 2022).

4.2 The black soldier fly, *Hermetia illucens*

The black soldier fly, *Hermetia illucens*, is a fly of the order Diptera, endemic in tropical and subtropical regions. Its life cycle passes through the stages of egg, larva, pupa and adult and is completed in a few weeks (Müller et al.,

2017). The stage used in animal feeding is the larva, which during its growth converts organic substrates, even poor ones, into a valuable biomass (Müller et al., 2017). This biomass is then used as a feed in two forms, either by feeding the larvae directly to the animals or by processing them to separate their main components, mainly proteins, lipids and chitin (Müller et al., 2017).

The larval stage is, together with the pupal stage, the most nutrient-rich, and its composition depends largely on the substrate on which the larva is reared (Lu et al., 2022). The protein content ranges from approximately 32 to 53% of dry matter and is characterized by a rich amino acid profile, with leucine and lysine representing the most abundant essential amino acids (Lu et al., 2022). Lipids account for approximately 18 to 33% of the dry matter, while saturated fatty acids constitute between 36 and 78% of the total fatty acid fraction (Lu et al., 2022). Among these, lauric acid (C12:0) is the predominant component, accounting for approximately 29 to 51% of the total fatty acids (Lu et al., 2022; Abd El-Hack et al., 2020). The larva also contains a polysaccharide called chitin, the major component of the arthropod exoskeleton, which is considered an indigestible fibre (Abd El-Hack et al., 2020; Lu et al., 2022). Chitin is a linear polymer made of N-acetyl-glucosamine units joined by β-(1,4) bonds, with a structure similar to that of cellulose (Lu et al., 2022). The average chitin content in the larva is approximately 6.2% of dry matter, with values ranging from 3.9 to 7.2% (Lu et al., 2022). Among minerals, the black soldier fly larva is particularly rich in calcium, which is its most abundant mineral and which the larva actively accumulates (Wang and Shelomi, 2017). Reported values for the larval meal are about 6.6 to 9.3% of calcium on a dry-weight basis, more than ten times the level of most other insects and higher than that of fish meal (Wang and Shelomi, 2017).

4.3 Use in animal nutrition

Black soldier fly larvae (BSFL) meal is used in animal feeding as a source of protein, to replace conventional protein feeds such as fish meal and soybean meal (Abd El-Hack et al., 2020). Most studies have investigated its use in poultry, pigs and fish, whereas its use in ruminants has been explored only more recently and is still less developed (Fukuda et al., 2022). The larvae can be fed to animals directly, as whole larvae, or they can be processed to separate their components (Müller et al., 2017). As a feed ingredient, the larvae are used in two forms, full-fat and defatted, which differ mainly in their fat content (Lu et al., 2022). The defatted meal is obtained by removing part of the fat, for example by mechanically pressing the larvae before milling (Wang and Shelomi, 2017). In full-fat form, the fat content ranges from about 29 to 52%, with an average of 35% (Lu et al., 2022). In the defatted meal the fat is much lower, about 7% of the dry matter on average (Lu et al., 2022). Protein content of the defatted meal varies widely, from approximately 22 to 66% of the dry matter depending on the defatting method (Lu et al., 2022).

In poultry, black soldier fly larvae meal has been studied as a replacement of soybean cake in diets of laying hens (Maurer et al., 2016). In layers, the substitution of soybean cake by partially or fully defatted larvae meal had no adverse effects on feed intake (Abd El-Hack et al., 2020). The complete replacement of soybean meal by BSF larvae meal, however, has in some cases reduced feed intake, by about 13% over 21 weeks, an effect that might be attributed to the colour and flavour of the larvae meal (Abd El-Hack et al., 2020). Black soldier fly larvae meal is also included in the diets of broilers and quails, where in one study it was used to replace part of the soybean oil of the diet (Abd El-Hack et al., 2020).

In a study on finishing pigs, BSFL meal was used as a replacement for fish meal, resulting in improved growth performance, carcass yield, and meat quality (Chia et al., 2021). Based on these findings, the authors concluded that black soldier fly larvae meal can effectively replace fish meal in pig diets (Chia et al., 2021). A study conducted on weaned piglets demonstrated that black soldier fly larvae meal can be included at levels of up to 10% of the diet without impairing growth performance (Biasato et al., 2019).

The use of black soldier fly larvae meal in ruminants has been investigated as a strategy to reduce the dependence on imported soybean meal (Braamhaar et al., 2025). Owing to their high protein content and the possibility of being produced from organic waste, black soldier fly larvae represent a sustainable alternative to soybean meal (Braamhaar et al., 2025). However, despite this potential, research on BSFL use in ruminants has only recently gained attention, and until a few years ago no *in vivo* studies had been conducted in cattle (Fukuda et al., 2022). A study conducted on dairy cows showed that replacing soybean meal with defatted black soldier fly larvae meal did not affect either milk yield or milk composition (Braamhaar et al., 2025). When used as a protein supplement for beef cattle fed low-quality forage, black soldier fly larvae stimulated forage intake, although the steers given the conventional supplement consumed slightly more (Fukuda et al., 2022).

4.4 Protein digestibility

When interpreting the protein digestibility of BSFL meal, two aspects must be considered. The first is that the crude protein, obtained by multiplying the total nitrogen by 6.25, overestimates the true protein of the larva, because part of the nitrogen comes from the chitin, a source of non-protein nitrogen

(Abd El-Hack et al., 2020). For this reason, a specific lower conversion factor of 4.67 has been proposed for black soldier fly larvae, since the factor 6.25 would overestimate their protein content (Smets et al., 2020). The second is that the chitin of the exoskeleton is not degraded by the animal's digestive enzymes, so the nitrogen within the chitin and any protein encapsulated by chitin are not digested and absorbed in the small intestine, which lowers protein digestibility (Crosbie et al., 2020).

In pigs, the standardised ileal digestibility (SID) of the crude protein of BSFL meal is about 81%, and that of lysine about 88%, with no difference between the full-fat and the defatted meal (Crosbie et al., 2020). In broilers, the ileal digestibility of the amino acids of BSFL meal is high, with an average standardised ileal digestibility coefficient of the amino acids of 0.84, and values of 0.85 for lysine, 0.90 for methionine, 0.91 for threonine and 0.87 for valine (Mahmoud et al., 2023). When digestibility is instead measured on the crude protein over the whole digestive tract, the value reported for the larvae meal in broilers is lower, only 51% (Abd El-Hack et al., 2020). The digestibility of the amino acids is higher because it reflects the true protein only, while the digestibility of the crude protein is lower because the crude protein is derived from the total nitrogen and therefore includes the non-protein nitrogen of the chitin, which is not digested. These values are not directly comparable, because they come from different methods, such as ileal or total-tract digestibility measured on amino acids or on crude protein. In ruminants, the protein digestibility of BSFL has been studied both at the ruminal level and at the intestinal level. In an in vitro rumen study, substituting soybean meal with black soldier fly larvae increased the neutral- and acid-detergent insoluble crude protein of the diets, that is the fraction of the crude protein bound to the fibre and not digestible, and lowered the ruminal ammonia concentration, indicating that less protein was degraded in

the rumen (Jayanegara et al., 2017). In a study conducted on goats, in which the rumen degradability was measured in situ and the intestinal digestibility in vitro, heat treatment increased the small intestinal digestibility of the protein of black soldier fly larvae from 47.8% in the untreated larvae to 67.8% when they were coated with cassava and treated at 140°C (Lu et al., 2024). This value was similar to that of the untreated full-fat soybeans, 70.3%, but remained lower than that of the heat-treated full-fat soybeans, which reached 78.8% (Lu et al., 2024). According to the authors, this difference depends on the anti-nutritional factors, since the main anti-nutritional factor of the larvae is the chitin, which resists heat treatment, whereas the anti-nutritional factors of the full-fat soybeans, such as the protease inhibitors, are more easily destroyed by heat (Lu et al., 2024). In a study on beef steers fed low-quality forage, using black soldier fly larvae as a protein supplement did not affect the digestibility of dry matter, organic matter or neutral detergent fibre compared with a conventional supplement; the authors concluded that the larvae can be an effective protein supplement for beef cattle consuming low-quality forage (Fukuda et al., 2022). In a trial on lactating dairy cows, the apparent total-tract digestibility of the crude protein was 67.8% and did not differ between the soybean meal diet and the defatted BSFL meal diet, and milk yield and composition were not compromised (Braamhaar et al., 2025).

4.5 Regulatory framework in the European Union

Insect-based products intended as feed can be grouped into three categories: live insects, processed animal proteins and derived products such as fat and chitin; insect protein is classified as processed animal protein (Żuk-Gołaszewska et al., 2022; Meijer et al., 2025). *Hermetia illucens* is one of

the seven insect species (together with *Musca domestica*, *Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus*, *Gryllodes sigillatus* and *Gryllus assimilis*) that Regulation (EU) 2017/893 lists among the species farmed in the European Union that fulfil the safety conditions for use in farmed and pet animal feed (Wang and Shelomi, 2017; Meijer et al., 2025). The use of animal proteins in feed is strictly regulated in the European Union. The main reason for these restrictions was to contain the spread of transmissible spongiform encephalopathies (TSEs), mainly bovine spongiform encephalopathy (BSE), which had been associated with the presence of infected material in animal feed resulting from the recycling of proteins of animal origin in feed (Meijer et al., 2023). The first regulations that limited the use of processed animal proteins in feed were introduced during the outbreak of these encephalopathies, and all feeds containing processed animal proteins were withdrawn from the market (Żuk-Gołaszewska et al., 2022). Before 2017, insect processed animal proteins could be fed only to fur animals and used in pet food (Meijer et al., 2025). In 2017 the processed animal proteins of the first seven farmed insect species were authorised as feed for aquaculture animals with Regulation (EU) 2017/893 (Meijer et al., 2025). In 2021 this authorisation was extended to pigs and poultry with Regulation (EU) 2021/1372, and *Bombyx mori* was added as an eighth species (Meijer et al., 2025). Ruminants remain the only group of food-producing animals to which farmed insect processed animal proteins cannot be fed, because of the permanent ruminant ban of Regulation (EC) No 999/2001, which prohibits the use of animal proteins in ruminant feed (Meijer et al., 2025; Meijer et al., 2023). An important exemption to this ruminant ban is the feeding of fish proteins to young ruminants, for example the use of fishmeal in calf milk replacers (Meijer et al., 2023). This exemption, however, concerns fish proteins and not insect processed animal

proteins, which remain not authorised for ruminants, whereas insect fat can be administered to all animals (Żuk-Gołaszewska et al., 2022). Similarly, live insects can legally be used as feed in the European Union, except for ruminants, and can be fed to pigs, poultry and aquaculture animals (Meijer et al., 2025). The substrates on which the larvae can be reared are restricted to products of non-animal origin or a limited set of animal products, and these restrictions eliminate the risk of prion contamination of the larvae (Wang and Shelomi, 2017).

4.6 Sustainability and competition with human food

Insect meal is mostly promoted as a substitute for fishmeal, which is associated with the depletion of forage fish, and for soybean meal, which is linked to deforestation (Biteau et al., 2026). In a life cycle assessment that compared black soldier fly production with soybean meal and fishmeal, the insect protein had greater environmental impacts than the protein from soybean meal or fishmeal, mainly because of the high energy consumption of the rearing and, in some cases, agro-product demands (Beyers et al., 2023). In that study, soybean meal and fishmeal outperformed the insect larvae in all the impact categories considered (Beyers et al., 2023). The environmental impact of insect farming depends mainly on two factors, which are the substrate on which insects are fed and the energy required for their rearing and processing (Biteau et al., 2026). High-quality substrates such as grains support a faster growth of the insects but often imply a higher environmental impact to be produced and can compete with their use as human food, whereas lower-quality substrates such as manure, household waste or potato peelings generally have a lower footprint but can slow the growth of the insects (Biteau et al., 2026). The lower-footprint substrates

such as manure and catering waste, however, cannot be used to rear insects for feed in the European Union, which limits the environmental benefit that black soldier fly farming could otherwise provide (Meijer et al., 2025). When insects are reared on feed-grade products and then used as feed, insect farming can increase the environmental footprint of our food system, because it adds a step in the food production chain (Biteau et al., 2026).

Black soldier fly larvae can be used both as a human food and as an animal feed (Lu et al., 2022). Consumers, however, prefer to use the larvae as animal feed rather than for direct human consumption, because of a certain psychological aversion to eating insects (Lu et al., 2022). Direct human consumption of BSFL is limited to a few areas, such as the Malaysian province of Sabah, where they are eaten by certain indigenous groups (Lu et al., 2022). At the industrial level, only a very small proportion of the investment in the insect sector, less than 5% in 2022, was directed to insect-based products intended for human consumption (Biteau et al., 2026).

Chapter 5: EXPERIMENTAL TRIAL

5.1 Introduction

The growth of the world population in the coming years will increase the demand for animal-source products, consequently requiring a considerable expansion of animal production (Nardone et al., 2010). This increase also carries environmental implications, among which nitrogen losses from animal production deserve particular attention. A large part of the nitrogen supplied by dietary protein is excreted by the animal and lost in the environment, and the amount lost through the faeces depends closely on how digestible the dietary protein is (Siegert et al., 2025). Increasing the digestibility of the protein sources used in animal feeding is therefore one of the main strategies to limit these losses.

In this context, there is growing interest in finding alternative protein sources. Among these, insects have attracted particular attention, and black soldier fly (*Hermetia illucens*) larvae especially are considered one of the most promising options (Shah et al., 2022). Their protein digestibility, however, has usually been assessed within a single animal category, making it difficult to establish how the same source behaves across ruminants and monogastrics, also because studies conducted in different species use different methods and metrics.

The aim of the present study was to evaluate and compare the protein digestibility of three conventional protein sources (roasted full-fat soybean, whole sunflower seed and herring meal) and black soldier fly (*Hermetia illucens*) larvae meal using in vitro systems simulating digestion in cattle, pigs and poultry to obtain comparable digestibility values and to assess whether black soldier fly larvae meal could represent a suitable protein source for animal feeding.

5.2 Materials and methods

The present study was carried out at the Department of Veterinary Sciences, University of Parma (Italy), between November 2025 and July 2026.

5.2.1 Experimental design

The four protein sources evaluated in this study were herring meal, roasted full-fat soybean, whole sunflower seed and black soldier fly (*Hermetia illucens*) full-fat larvae meal. The insect meal was purchased from Société APPI (Pont St Martin, France), whereas the three conventional sources were supplied by two commercial feed companies, Menozzi Mangimi (Montechiarugolo, Parma, Italy) and Neviani Mangimi (Montecchio Emilia, Reggio Emilia, Italy). All the ingredients were ground with a Retsch SK mill (Bauknecht, Stuttgart, Germany) to pass a 1-mm mesh screen and stored refrigerated until analysis.

The chemical composition of the four substrates is reported in Table 1.

Table 1 Chemical composition of the four protein sources (herring meal, roasted full-fat soybean, whole sunflower seed and black soldier fly larvae meal) expressed on a dry matter basis

Nutrients, %DM	Herring	Soybean	Sunflower	BSFL
Dry matter	92.4	91.7	94.1	94.2
Crude protein	76.12	36.60	19.06	31.14
Ether extract	4.67	21.27	47.20	41.81
Ash	19.67	6.01	3.02	4.94
Neutral detergent fibre	3.77	23.44	42.06	42.24
Acid detergent fibre	1.45	12.28	28.81	9.12
Acid detergent lignin	0.89	5.67	14.69	2.68

Each source was sampled in duplicate, and each sample was run in triplicate, for a total of six aliquots per source in each digestion phase. The four sources were incubated in three in vitro digestion systems reproducing the gastrointestinal conditions of cattle, pigs and poultry. The ruminant system consisted of a ruminal phase and an abomasal and intestinal phase, providing the ruminal degradability of dry matter (DM) and crude protein (CP) and the intestinal digestibility of DM and CP. The swine system included a gastric and intestinal phase, providing the digestibility of DM and CP. The poultry system consisted of a crop phase, which provided the solubilization of DM and CP in the crop, and of a gastric and intestinal phase, which provided the digestibility of DM and CP. In each phase of every system a blank was included to correct the results.

Bovine Digestibility Assay

Bovine digestibility was assessed through a three-step procedure reproducing the ruminal, abomasal and intestinal phases. The ruminal phase was performed according to Goering and Van Soest (1970), while the abomasal and intestinal phases followed the method of Ross et al. (2013). For the ruminal phase, 0.5 g of each substrate were weighed into conical flasks and 40 mL of Van Soest buffer were added. Rumen fluid was collected from different cows at the slaughterhouse, stirred, and filtered through four layers of cheesecloth under continuous flushing with CO_2. Ten mL of filtered rumen fluid was then inoculated at a 1:4 ratio in each flask, followed by incubation at 39 °C under CO_2 flushing for 16 h. At the end of the ruminal phase, the flasks used to assess ruminal degradability were removed from the water bath and refrigerated, while the others continued to the following phases. For the abomasal phase, the ruminal digesta were acidified to pH 1.9 with 3 M HCl, and 2 mL of acid pepsin solution (224.1 Worthington U/mL),

prepared in 0.013 M HCl, was added. The flasks were then incubated at 39 °C for 1 h without CO_2 flow. For the intestinal phase, the pH was raised to 5 with 2 M NaOH, and 10 mL of an enzyme mixture in phosphate buffer 1.8 M were added. The enzyme solution included trypsin (PANREAC, code A4148, from porcine pancreas, 250 USP U/mg, 750 BAEE U/mg, Barcelona, Spain), chymotrypsin (MERCK, code 1.02307.0001, from bovine pancreas, activity 350 BTEE U/mg, Darmstadt, Germany), α-amylase (SIGMA, code 10069, from *Bacillus subtilis*, activity 380 U/mg, Sigma-Aldrich, St. Louis, MO, USA), bile salts (MILLIPORE, code B3883-100, from bovine**,** Darmstadt, Germany), and lipase (MP Biomedicals, code 102189-80, from porcine pancreas, activity 10 USP U/mg, Santa Ana, CA, USA). The flasks were then incubated at 39 °C for 24 h without CO_2 flow.

At the end of the ruminal and of the intestinal phase, the undigested residue was filtered through tared borosilicate crucibles (Robu Glass Filter–ROBU H3, Borosilicate 3.3, 30 mL, Por. 2, Hattert, Germany), which were then rinsed three times with distilled water, dried overnight at 103°C, cooled in a desiccator and weighed to determine the residual DM. The residue in the crucible was then collected and analysed for the N content by the Dumas method as described by Mihaljev et al. (2015) to determine the residual CP.

Swine Digestibility Assay

Swine digestibility was assessed following the procedure described by Boisen and Fernández (1997), reproducing the gastric and intestinal phases. About 0.5 g of each substrate was weighed into conical flasks. For the gastric phase, 25 mL of phosphate buffer (0.1 M, pH 6.0) was added, followed by 10 mL of 0.2 M HCl, and the pH was adjusted to 2.0 with 1 M HCl or 1 M NaOH. Then, 1 mL of a pepsin solution, prepared in 0.2 M HCl and containing 25 mg of pepsin (porcine, P-7000, CAS no.9001-75-6, Sigma-

Aldrich, St. Louis), was added to each flask. The flasks were closed and incubated at 39 °C for 2 h. After incubation, for the intestinal phase, 10 mL of phosphate buffer (0.2 M, pH 6.8) and 5 mL of 0.6 M NaOH were added, and the pH was adjusted to 6.8 with 1 M HCl or 1 M NaOH. Then, 0.5 mL of a pancreatin solution (100 mg/mL Pancreatin 8 USP, P-7545, CAS no. 8049-47-6, Sigma-Aldrich, St. Louis, MO, USA) prepared in 0.6 M NaOH was added, and the flasks were incubated at 39 °C for 4 h.

At the end of the intestinal phase, the undigested residue was filtered through tared borosilicate crucibles (Robu Glass Filter–ROBU H3, Borosilicate 3.3, 30 mL, Por. 2, Hattert, Germany), which were then rinsed three times with distilled water, dried overnight at 103°C, cooled in a desiccator and weighed to determine the residual DM. The residue in the crucible was then collected and analysed for the N content by the Dumas method as described by Mihaljev et al. (2015) to determine the residual CP.

Poultry Digestibility Assay

Poultry digestibility was assessed following the procedure described by Witten and Aulrich (2022), reproducing the crop, gastric and intestinal phases. About 1.5 g of each substrate was weighed into conical flasks. For the crop phase, 7.5 mL of phosphate buffer (1 M, pH 6.0) were added and the flasks were incubated for 30 min at 41 °C to simulate the conditions of the crop. For the gastric phase, 1.5 mL of a pepsin solution (0.01 g pepsin/mL, Merck No. 7190, 2000 FIP-U/g), freshly prepared in 0.2 M HCl, was added, and the pH was adjusted to 2.6 with 5 M HCl and 5 M NaOH. The flasks were then incubated for 135 min at 41 °C. For the intestinal phase, 0.0375 mL of 0.6 M NaOH and 1.5 mL of a pancreatin solution (0.05 g pancreatin/mL, Merck No. P1750, 4×USP, freshly prepared in phosphate buffer 0.2 M, pH 6.8) were added, and the pH was adjusted to 6.4 with 5 M

NaOH and 5 M HCl. The flasks were then incubated for 120 min at 41 °C. During all incubations, the flasks were manually shaken approximately every 15 min.

At the end of the crop and of the intestinal phase, the undigested residue was filtered through tared borosilicate crucibles (Robu Glass Filter–ROBU H3, Borosilicate 3.3, 30 mL, Por. 2, Hattert, Germany), rinsed three times with distilled water, dried overnight at 103 °C, cooled in a desiccator and weighed to determine the residual DM. The residue in the crucible was then collected and analysed for the N content by the Dumas method as described by Mihaljev et al. (2015) to determine the residual CP.

5.2.2 Chemical analysis

The four protein sources were analysed with the following methods: DM was determined by oven-drying at 103 °C overnight, ash content was determined gravimetrically after incineration at 550 °C for 4 h, and the ether extract was determined by the Soxhlet method, following the Commission Regulation (EC) No 152/2009 (EC, 2009). Neutral detergent fibre (aNDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined sequentially, using a heat-stable amylase for aNDF and without the addition of sodium sulphite (Robertson and Van Soest, 1981). The N content was determined by combustion at 900 °C in excess of oxygen using a Dumatherm (Gerhardt GmbH & Co, Königswinter, Germany), as described by Mihaljev et al. (2015), and the CP content was calculated by multiplying the N value by a conversion factor of 6.25 for the conventional sources and of 4.76 for the black soldier fly larvae meal, as this factor excludes the N bound to chitin (Janssen et al., 2017).

5.2.3 Calculations

For each phase, dry matter digestibility (DMD) and crude protein digestibility (CPD) were calculated as

$$DMD\ (\%) = 100 - [(r - b) / (w \times DM)] \times 100$$

$$CPD\ (\%) = 100 - [((100 - DMD) \times CPr / 100) / (CPi / 100)]$$

where r is the weight of the undigested residue, b is the weight of the blank residue, w is the weight of the sample, DM is the dry matter content of the sample, CPr is the crude protein content of the residue (residual N multiplied by the conversion factor), and CPi is the crude protein content of the sample. These values are reported as “ruminal DMD” and “rumen degradable protein (RDP)” in the ruminal phase, as “crop DM solubilisation” and “soluble dietary protein (sDP)” in the crop phase, and as “DMD” and “CPD” in the intestinal phase.

5.2.4 Statistical analysis

Statistical analysis was performed using SPSS for Windows software package (version 30; SPSS Inc., Chicago, IL). For each trait, differences among substrates within each animal model were evaluated separately through the univariate procedure of the General Linear Model, using substrate as a fixed effect and aliquot as a random effect. Differences among animal species were then evaluated for each trait through a mixed model, using substrate and species as fixed effects and aliquot as a random effect. In both analyses, pairwise comparisons were performed using the Bonferroni post hoc test. Differences were considered significant at $P < 0.05$.

5.3 Results and discussion

5.3.1 Pre-gastric degradation and solubilisation

Table 2 Ruminal dry matter digestibility (ruminal DMD, %) and rumen degradable protein (RDP, % of crude protein) of the four protein sources incubated in the bovine in vitro system

	Herring	Soybean	Sunflower	BSFL	SEM	P_value
Ruminal DMD, % DM	31^{B}	70.2^{D}	51.8^{C}	25.3^{A}	3.77	<0.001
RDP, % CP	37.2^{B}	77.6^{C}	87.8^{D}	26.4^{A}	5.56	<0.001

At the end of the ruminal phase, the four protein sources differed in both DMD and RDP. The ruminal DMD was highest in roasted full-fat soybean (70.2%), followed by whole sunflower seed (51.8%), herring meal (31.0%) and BSFL meal (25.3%). RDP was the highest in whole sunflower seed (87.8%), followed by roasted full-fat soybean (77.6%), herring meal (37.2%) and BSFL meal (26.4%). The two oilseeds, soybean and sunflower, were therefore the most degraded in the rumen, while BSFL and herring showed the lowest ruminal degradability among the four sources, both for DM and for CP.

The low ruminal DMD of herring meal can be explained by its composition, since its DM is made up mostly of CP and ash, the latter being essentially undegradable. As the protein of herring meal behaves as a bypass protein that is only partially degraded in the rumen, the largest fraction of its DM resists ruminal fermentation, which results in a low ruminal DMD (Harstad and Prestløkken, 2001). The low RDP is in line with this behaviour, since only a small part of the CP of herring meal is degraded in the rumen, so that a large fraction escapes ruminal fermentation and reaches the small intestine as RUP.

Roasted full-fat soybean showed the highest ruminal DMD among the four sources and a high RDP. The high ruminal DMD can be explained by its composition. A large part of its DM is made up of protein, NDF and ether extracts, that were extensively degraded in the rumen. Moreover, the CP was highly degradable in the rumen. For these reasons most of its DM, including the fat which is solubilizable and thus pass through the crucible during the analysis, is readily fermented in the rumen, which explains the high ruminal DMD. The RDP value is higher than what would be expected for a roasted full-fat soybean, which is heat-treated precisely to increase the proportion of protein that escapes ruminal degradation, up to about 35 to 42% (INRAE-CIRAD-AFZ, 2024), corresponding to a rumen degradable protein of roughly 58 to 65%. The RDP measured here, 77.6%, is above this range, and is consistent with the N digestibility in ruminants of 78.2%. Compared with the raw sunflower seed, however, roasted soybean still showed a lower RDP, which is in the direction expected from the roasting.

Whole sunflower seed showed the highest RDP among the four sources and a moderate ruminal DMD, lower than that of roasted full-fat soybean. The high RDP can be explained by the fact that sunflower seed receives no heat treatment, so its protein is not protected and is extensively degraded in the rumen. This value is in close agreement with the rumen degradability of about 82% reported for whole sunflower seed (INRAE-CIRAD-AFZ, 2024). Despite this high protein degradation, the moderate ruminal DMD is explained by the composition of the seed, which is rich in strongly lignified hull fibre that is poorly degradable (Selvaraj and Purushothaman, 2004) and in fat. For these reasons a large part of its DM resists ruminal degradation, and its ruminal DMD is lower than that of soybean even though its protein is more extensively degraded.

The BSFL meal was the least degraded source in the rumen, showing the lowest values among the four sources both for ruminal DMD and for RDP. The low ruminal DMD of BSFL meal, in line with the value reported by Bonomini et al. (2026), can be explained by its composition, which is rich in lipid and in chitin and poor in fermentable carbohydrate. The high lipid content is not fermented in the rumen, and the chitin, the main constituent of the insect exoskeleton, is poorly fermented by the rumen microbiota because of its compact structure and extensive hydrogen bonding (Bonomini et al., 2026). The low RDP indicates that the protein of BSFL was also poorly degraded in the rumen. This can be attributed to the fact that a large part of the CP of the larvae is bound to the fibre fraction, which in insects corresponds largely to the chitin of the exoskeleton. This bound protein is not readily accessible to the rumen microbiota, so that part of it is protected from ruminal degradation (Jayanegara et al., 2017). The rumen, however, exhibits some activity towards chitin, since chitinolytic micro-organisms able to attack it have been reported in the rumen environment (Fukuda et al., 2022).

Table 3 Crop dry matter solubilisation (%) and soluble dietary protein (sDP, % of crude protein) of the four protein sources in the crop phase of the in vitro poultry system

	Herring	Soybean	Sunflower	BSFL	SEM	P_value
Crop DM solubilisation, % DM	30.8^{B}	30.3^{B}	21.5^{A}	18.3^{A}	1.26	<0.001
sDP, % CP	34.9^{C}	25.6^{B}	44.6^{D}	17.5^{A}	2.17	<0.001

At the end of the crop phase, the four protein sources differed in both DM solubilisation and sDP. Crop DM solubilisation was higher in herring meal (30.8%) and roasted full-fat soybean (30.3%) compared to whole sunflower seed (21.5%) and BSFL meal (18.3%). The sDP was highest in whole

sunflower seed (44.6%), followed by herring meal (34.9%), roasted full-fat soybean (25.6%) and BSFL meal (17.5%). The BSFL meal showed the lowest sDP and one of the lowest values of DM solubilisation, and was therefore the least solubilised source in the crop.

The high crop DM solubilisation of herring meal can be related to its ash content, which is the highest of the four sources (Table 1). This mineral fraction may have been partially solubilised under the conditions of the essay, as the acidic pH of the phosphate buffer, together with the incubation temperature, could favour the partial dissolution of these minerals. The solubilised DM also includes part of the protein, reflected in the sDP, which is unlikely to be native protein, since the cooking applied during fish meal production strongly reduces the solubility of the native protein (Nguyen et al., 2022); in addition, a fraction of free amino acids may have passed into solution.

Roasted full-fat soybean reached a crop DM solubilisation similar to that of herring meal, even though its soluble fraction has a different origin. Indeed, soybean has a much lower ash content than herring meal (Table 1), so its soluble DM comes from organic components rather than from a soluble mineral fraction. These organic components are probably sugars and non protein nitrogen, including aminoacids and peptides present in the soybean seed, which represent about 9% of the DM (INRAE-CIRAD-AFZ, 2024) and are readily soluble.

In whole sunflower seed, the low crop DM solubilisation can be explained by its composition, since its DM is dominated by fat and by the strongly lignified fibre of its hull (Table 1), which do not dissolve in the buffer, so that only a small part of the DM passes into solution. Its high sDP, in contrast, is because sunflower seed receives no heat treatment, so that its protein has

not undergone the loss of solubility caused by thermal processing, and therefore retains a higher solubility.

In BSFL meal, the low crop DM solubilisation may be attributed to its composition, since its DM is rich in fat (Table 1) and contains chitin from the exoskeleton, both of which are poorly soluble in the buffer, resulting in only a small fraction of the DM being solubilised. The low sDP may be due to the substantial fraction of insect protein bound to chitin, which limits its solubility and consequently the amount of protein released into solution during the crop phase (Jayanegara et al., 2017).

5.3.2 Total dry matter digestibility

Table 4 Total dry matter digestibility (DMD, % of dry matter) of the four protein sources at the end of the complete in vitro digestion in cattle, poultry and swine

DMD,%DM

	Herring	Soybean	Sunflower	BSFL	SEM	P_value
Cattle	67.7^{bA}	90.0^{cB}	68.3^{cA}	62.3^{bA}	2.55	<0.001
Poultry	33.8^{aAB}	41.7^{aBC}	28.2^{aA}	48^{aC}	1.85	<0.001
Swine	72.9^{bB}	66.5^{bB}	45.8^{bA}	70.9^{cB}	2.36	<0.001
SEM	4.71	4.86	4.04	2.65		
P_value	<0.001	<0.001	<0.001	<0.001		

Uppercase letters indicate differences between feeds
Lowercase letters indicate differences between species

The total DMD was significantly affected by the protein source within each species and by the species within each source.

In cattle, roasted full-fat soybean had the highest DMD (90.0%), while herring meal, whole sunflower seed and BSFL meal were similar for this parameter. In poultry, BSFL meal had the highest DMD (48.0%), together with soybean (41.7%), while whole sunflower seed and herring meal showed lower values (28.2% and 33.8% respectively). In swine, whole sunflower

seed had the lowest DMD (45.8%), while the other three sources had similar digestibility.

Herring meal was digested to a similar extent in cattle (67.7%) and swine (72.9%), and to a significantly lower extent in poultry (33.8%). Roasted full-fat soybean was most digestible in cattle (90.0%), followed by swine (66.5%) and then poultry (41.7%), with the three species differing significantly. Whole sunflower seed followed the same pattern, being highest in cattle (68.3%), intermediate in swine (45.8%) and lowest in poultry (28.2%). The BSFL meal was instead more efficiently utilised in swine (70.9%), than cattle (62.3%) and poultry (48.0%).

The BSFL was the only source that was digested to a greater extent in a monogastric, the swine, than in ruminants. In swine its total DMD (70.9%) was in fact similar to that of herring meal and soybean, and in poultry it was the most digestible of the four sources. The two main dry matter components of BSFL meal, fat and protein, together accounted for more than 70% of the DM (Table 1). The protein is readily digested by the enzymes of the gastric and intestinal phases. The fat is also largely digested, as indicated by the total DMD being considerably higher than the protein content of the larvae, suggesting that a substantial proportion of the digested DM is attributable to fat rather than only protein. On the other side the chitin of the exoskeleton is not attacked by these enzymes (Crosbie et al., 2020), with chitin therefore remaining the main undigested fraction of the larvae. This can be explained by the action of enzymes and bile salts during the abomasal and intestinal phases, which promote the digestion of both fat and protein in the larvae, thereby explaining the markedly higher total DMD compared with their low ruminal degradation (Bonomini et al., 2026).

Whole sunflower seed was among the least digestible sources in all the three species, and it was the lowest in poultry and in swine. Its DM contains a large

proportion of strongly lignified fibre from the hull (Table 1; Selvaraj and Purushothaman, 2004), and lignin is resistant both to microbial fermentation and to the animal's digestive enzymes, making this fraction indigestible throughout the digestive tract of all three species and thereby lowering the total DM digestibility of the seed.

Across the three species, poultry showed the lowest DM digestibility for every source. Since the retention time of digesta determines how long the feed is exposed to digestive enzymes, a shorter retention time limits the time available for digestion. The incubation times used in the poultry in vitro method are shorter than those adopted for the other two species and were established on the basis of the shorter retention times in the chicken digestive tract (Witten and Aulrich, 2022). Thus, the lower DM digestibility observed in poultry may reflect the shorter period available for digestion.

Roasted full-fat soybean was highly digestible in all species and reached the highest value in cattle (90.0%), in agreement with its low content of poorly degradable fibre. Generally, cattle showed the highest digestibility for both plant oilseeds, soybean and sunflower, probably in relation to the capacity of rumen microbiota to ferment plant constituents that enzymes of monogastric animals cannot break down.

5.3.3 Total crude protein digestibility

Table 5 Total crude protein digestibility (CPD, % of crude protein) of the four protein sources at the end of the complete in vitro digestion in cattle, poultry and swine

CPD, %CP

	Herring	Soybean	Sunflower	BSFL	SEM	P_value
Cattle	74.5^{bA}	98.2^{bB}	95.1^{cB}	79.1^{cA}	2.15	<0.001
Poultry	45.9^{aA}	70.3^{aB}	78^{bB}	49.3^{aA}	2.95	<0.001
Swine	71.8^{bBC}	73.8^{aC}	42.7^{aA}	63.4^{bB}	2.75	<0.001
SEM	3.22	3.20	5.36	3.07		
P_value	<0.001	<0.001	<0.001	<0.001		

In cattle, roasted full-fat soybean and whole sunflower seed had the highest CPD (98.2% and 95.1%), while herring meal (74.5%) and BSFL meal (79.1%) showed lower and similar values. In poultry, soybean and sunflower were again the most digestible (70.3% and 78.0%), and herring meal (45.9%) and BSFL meal (49.3%) the least. In swine, whole sunflower seed had the lowest CPD (42.7%). Among the other three sources, soybean (73.8%) was more digestible ($P = 0.020$) than BSFL meal (63.4%), while herring meal (71.8%) did not differ from either of them.

Herring meal was digested to a similar extent in cattle (74.5%) and swine (71.8%), and to a significantly lower extent ($P < 0.001$) in poultry (45.9%). Roasted full-fat soybean reached its highest CPD in cattle (98.2%), while poultry (70.3%) and swine (73.8%) showed lower and similar values. Whole sunflower seed showed the widest variation across species, being highest in cattle (95.1%), intermediate in poultry (78.0%) and lowest in swine (42.7%), with the three species differing significantly ($P < 0.001$). The BSFL meal was most digestible in cattle (79.1%), followed by swine (63.4%) and then poultry (49.3%), again with the three species differing significantly ($P < 0.001$).

Cattle showed the highest CPD across all four sources. Specifically, cattle had the highest CPD for soybean, sunflower, and BSFL, whereas for herring meal, cattle together with swine showed the highest values. This higher CPD in cattle reflects the fact that CP undergoes two successive processes of degradation and digestion, being subjected to microbial degradation in the rumen and subsequently to enzymatic digestion in the abomasum and small intestine. In contrast, in monogastric species, CPD reflects the enzymatic digestion only.

The two plant protein sources -soybean and sunflower- reached CPD values in cattle that were close to the complete digestion (98.2% and 95.1%, respectively). In sunflower seed, the lignin of the hull limits the digestibility of DM but does not substantially affected protein digestibility, as most of the protein is contained in the kernel and remains accessible. The high CPD observed for both oilseeds therefore indicates that their protein is almost completely digested in cattle. In raw sunflower seed, the high solubility of the protein may further contribute to its extensive degradation in the rumen (INRAE-CIRAD-AFZ, 2024). For soybean, this almost complete CPD is in agreement with the high digestibility of its protein, which is extensively degraded in the rumen and whose undegraded fraction is digested in the intestine at about 82% (INRAE-CIRAD-AFZ, 2024).

For BSFL meal, the CPD differed significantly among the three species, being highest in cattle (79.1%), intermediate in swine (63.4%) and lowest in poultry (49.3%). In the insect, part of the protein is bound to chitin, which is only partly degraded along the digestive tract and keeps this protein fraction less accessible (Bonomini et al., 2026). The higher CPD in cattle can be related to the ruminal phase, where, as described for dry matter, part of the chitin is degraded by the rumen microbiota before the intestinal phase, so that more of the bound protein is released and digested. In the two

monogastric methods this step is absent and only the animal's own enzymes act on the sample, which leaves a larger part of the chitin-bound protein undigested and results in a lower CPD. The low value observed in poultry may also be partly explained by the shorter incubation times adopted in the in vitro method, which were designed to reflect the retention times in the chicken digestive tract.

The CPD of herring meal was similar in cattle (74.5%) and swine (71.8%), with no significant difference between the two, and significantly lower in poultry (45.9%). In cattle and swine these values are in line with the recognised high protein quality of fish meal (Cho et al., 2011), and in swine herring meal was among the most digestible sources.

For sunflower seed, CPD was very high in cattle (95.1%) and high in poultry (78.0%), and markedly lower in swine (42.7%), the three species differing significantly ($P < 0.001$). The high values in cattle and poultry are in line with the good digestibility of sunflower seed protein, which is contained in the kernel and well accessible. The low CPD value obtained in swine, by contrast, does not agree with the standardised ileal nitrogen digestibility of whole sunflower seed reported for pigs, which is about 82% (INRAE-CIRAD-AFZ, 2024). This low value was also associated with a high variability among replicates (SEM 5.36, the highest in the table). This is probably also related to the physical characteristics of the ground sample that appeared, because of the lignified hull, more dishomogeneous and tended partially to float on the digestion fluids.

5.4 Conclusions

The aim of this study was to assess whether black soldier fly (*Hermetia illucens*) larvae meal can be a suitable alternative protein source for cattle,

pigs and poultry, by comparing its protein digestibility with that of three conventional sources (roasted full-fat soybean, whole sunflower seed and herring meal), using in vitro methods specific to each animal.

In each species, crude protein digestibility of BSFL meal was not significantly different from that of herring meal, indicating a protein digestibility comparable to that of a conventional animal protein source. Based on these results, BSFL meal can be considered a possible substitute for herring meal in all three species. Its CPD was, however, lower than that of soybean in all species, and lower than that of sunflower seed in cattle and poultry. In cattle, soybean and sunflower seed reached CPD values close to complete digestion, well above that of BSFL meal. Therefore, based on protein digestibility, BSFL meal cannot be considered a suitable substitute for either of the two oilseeds.

The lower digestibility of BSFL meal protein is related to the chitin of the larval exoskeleton, which binds part of the protein and resists breakdown. This limitation was observed in all three species, despite being less marked in cattle, where the ruminal microbiota degrades part of the chitin before the intestinal phase. However, this additional microbial degradation did not completely overcome the limitation, as BSFL protein remained less digestible than soybean and sunflower seed even in cattle. In swine and poultry, where the in vitro method involved only enzymatic digestion, chitin had an even more pronounced effect on protein digestibility.

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