# CHAPTER II
# LITERATURE REVIEW

## 2.1 Black soldier fly (BSF (*Hermetia illucens* L.; Diptera: Stratiomyidae))

BSF is a saprophytic insect that primarily feeds on organic wastes such as plant residues, animal manure, and waste, food waste, agricultural byproducts, or straw (Banks et al., 2014; Lalander et al., 2013; Nguyen et al., 2015; Zheng et al., 2012). BSF is an excellent candidate for human and animal protein sources, and the utilization of organic waste can help to reduce pollution (Erickson et al., 2004; Paengkoum, 2010; Purba et al., 2021). In the process of degrading waste, BSF converts organic waste into amino acids, peptides, proteins, oils, chitin, and vitamins, thereby controlling certain harmful bacteria (such as Salmonella and Escherichia coli) and pests, and are also used in medicine and chemical and various animal feeds (mainly pets, pigs and poultry) (Erickson et al., 2004; Liu et al., 2008).

BSF originated in the South American savannah and is widely distributed in temperate, subtropical, and tropical regions, with an optimum temperature range of 25°C to 30°C (James, 1935). Due to their lack of resistance to cold, they cannot survive in northwestern Europe and regions with temperatures below 5°C (Spranghers Noyez et al., 2017). BSF is one of five genera in the subfamily Hermetiinae of the order Diptera (Woodley, 2011). The other four genera are Patagiomyia, Chaetosargus, Notohermetia, and Chaetohermetia; *Hermetia illucens* is the most widespread of all species (Singh and Kumari, 2019). It is a large, slender black species with three segments-head, thorax, and bell-with brownish wings and tentacles projecting from the head (Üstüner et al., 2003). There are five segments on the abdomen with white spots. Males are longer than females but have smaller end genitals and wings. Females have body lengths between 12 and 20 mm and wings between 8 and 14.8 mm (Üstüner et al., 2003). Their life cycle has five stages: egg, larva, pupa, pre- and adult. The larval and pupal stages are the most nutrient-rich and largely depend on the quality of food, with about 18–33% fat and 32–53% protein (Chippindale et al.,

2004; St-Hilaire et al., 2007; Yu et al., 2009). BSF has a lifespan of approximately 20-22 days, with a pupa for the first 6–8 days and an adult metamorphosis for the last 14 days. Adult worms have no mouth, digestive system, or stinger, pose no threat to other organisms (Park, 2016), and have no affinity for the human body and fresh food. Therefore, they also do not serve as vectors for disease transmission (Sheppard et al., 2002).

The bioconversion rate is one of the important indicators of waste efficiency used to treat BSF (Gold et al., 2020; Lalander et al., 2019), and the biotransformation rate depends on many factors, such as the concentration of digestible nutrients, protein, fat, fiber, pH, feeding rate (Banks et al., 2014), density and water content of substrates, etc. (Banks et al., 2014; Dortmans et al., 2017). The ideal moisture content is between 60% and 80%, with a lower limit of about 40% (Bortolini et al., 2020). The management requirements of livestock manure around the world are getting higher and higher (Paengkoum et al., 2019). Supplementing BSF in livestock feed can reduce the excretion of manure by 60% (Siddiqui et al., 2022). Moreover, the larvae can also decompose more than 50% of chicken manure, and convert it into high-quality amino acids, protein, and fat for animal feed, reducing the cost of breeding (Newton et al., 2005). At the same time, BSF nutrients are also rich in minerals and chitin and have antioxidant and immune-boosting properties.

## 2.2 Nutritional Value of BSF

### 2.2.1 Regular Nutrition Facts

In animal feed, there are currently two types of BSF: defatted and full fat, with the primary difference being in fat and saturated fatty acid content. Table 1 shows its nutritional value. The average crude protein content of BSF was 414.7 g/kg, ranging from 216 g/kg (Yildirim-Aksoy et al., 2020) to 655 g/kg (Schiavone Cullere et al., 2017), which was lower than a conventional soybean meal (CSBM) (494.4 g/kg) and fish meal (675.3 g/kg) (Council, 2012). The protein content of full-fat BSF is relatively similar, but the protein content after defatting is very different (from 216 g/kg to 655 g/kg), which may be related to the method of defatting, such as the irreversible damage to the protein caused by high temperatures. In full-fat BSF, the fat content

ranged from 294 g/kg (Onsongo et al., 2018) to 515.3 g/kg (Tyshko et al., 2021), with an average of 353.2 g/kg; these were both higher than CSBM (14 g/kg) and fish meal (103.6 g/kg) (Council, 2012). The average fat level after defatting (69.2 g/kg) was also higher than CSBM. BSF contained ash at an average of 82.4 g/kg, ranging from 27 g/kg (Spranghers Noyez et al., 2017) to 132 g/kg (Onsongo et al., 2018), higher than CSBM (71.9 g/kg) and lower than a fish meal (171.5 g/kg) (Council, 2012). The average level of crude fiber was 95.4 g/kg, ranging from 41 g/kg (Spranghers Noyez et al., 2017) to 213 g/kg (Onsongo et al., 2018), which was lower than CSBM (74.3 g/kg), but higher than a fish meal (2.6 g/kg) (Council, 2012). The average content of chitin was 61.7 g/kg, ranging from 38.7 to 72.1 g/kg. The active ingredient of chitin is chitosan, which is another important polysaccharide in addition to cellulose. Chitin (linear polymer of -(1-4)N-acetyl-d-glucosamine units) and cellulose (linear polymer of -(1-4)d-glucopyranose units) have similar molecular structures (Finke, 2007). Chitin is considered an indigestible fiber, but it can improve the immune function of animals (Li et al., 2013; Swiatkiewicz et al., 2015). The variation of crude fiber content in BSF may be related to developmental stages, and the closer to metamorphic adults, the higher the fiber content (H Wang et al., 2020). Therefore, different research results produced different levels of chitin content.

**Table 2.1** Regular nutrition facts of BSF (g kg-1 dry matter basis).

| | BSF | | | | | | | | | CSBM | FM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Type** | **FF** | **DF** | **DF** | **FF** | **FF** | **FF** | **FF** | **FF** | **DF** | | |
| Crude protein | 431.0 | 655.0 | 216.0 | 411.0 | 439.0 | 350.0 | 401.0 | 275.4 | 554.2 | 494.4 | 675.3 |
| Crude fat | 386.0 | 46.0 | 63.0 | 301.0 | 294.0 | 298.0 | 325.0 | 515.3 | 98.5 | 14.0 | 103.6 |
| Crude fiber | 41.0 | | 70.0 | | 213.0 | 79.0 | | | 74.0 | 74.3 | 2.6 |
| Ash | 27.0 | 93.0 | 93.0 | 93.0 | 132.0 | 53.0 | 104.0 | 65.9 | 81.0 | 71.9 | 171.5 |
| Chitin | 67.0 | 69.0 | | | | | | 38.7 | 72.1 | | |

CSBM = conventional soybean meal, FM = fish meal, FF = full-fat, DF = defatted.

### 2.2.2 Amino Acid Profile

Both defatted and full-fat BSF has a rich amino acid profile and are thus considered a more sustainable protein source than CSBM or fish meal (Crosbie et al., 2020). The amino acid profile of BSF is shown in Table 2.2. The most abundant essential amino acids were leucine (average 44.6 g/kg, from 27.8 g/kg to 78.3 g/kg),

lysine (average 38.8 g/kg, from 23.0 g/kg to 68.2 g/kg), and valine (average 40.1 g/kg, ranging from 28.2 g/kg to 67.9 g/kg). These three amino acid contents are higher than those of soybean meal, and even the valine content is higher than that of fish meal (Council, 2012). The least abundant essential amino acids are methionine and tryptophan, which are comparable to soybean meal and are much lower than fish meal (Council, 2012). The content of histidine ranged from 9.8 g/kg to 48 g/kg, and the content of isoleucine ranged from 17.7 g/kg to 48 g/kg, which was slightly higher than soybean meal and fish meal (Council, 2012). The content of phenylalanine ranged from 16.4 g/kg to 77.6 g/kg, and the content of threonine ranged from 16.2 g/kg to 45 g/kg, which is basically the same as soybean meal and fish meal. Arginine and histidine are lower than soybean meal and fish meal (Council, 2012).

**Table 2.2** Amino acid composition of BSF (g kg−1 dry matter basis).

| | BSF | | | | | | | CSBM | FM |
|---|---|---|---|---|---|---|---|---|---|
| **Indispensable amino acids** | | | | | | | | | |
| **Type** | **FF** | **DF** | **FF** | **FF** | **FF** | **FF** | **FF** | | |
| Arginine | 19.9 | 20.7 | 21.1 | 54.7 | 62.0 | 21.9 | 18.7 | 35.7 | 41.0 |
| Histidine | 13.8 | 16.3 | 13.5 | 32.5 | 48.0 | 9.8 | 13.7 | 14.2 | 15.4 |
| Isoleucine | 19.1 | 24.0 | 17.7 | 47.3 | 48.0 | 19.1 | 20.6 | 22.1 | 27.3 |
| Leucine | 30.6 | 36.7 | 27.8 | 78.3 | 77.0 | 32.1 | 29.4 | 38.6 | 47.7 |
| Lysine | 23.0 | 25.2 | 28.1 | 68.2 | 74.0 | 27.2 | 25.9 | 31.1 | 48.7 |
| Methionine | 7.1 | 8.56 | 8.0 | 21.2 | 6.0 | 6.0 | 7.1 | .6.8 | 18.5 |
| Phenylalanine | 16.4 | 21.8 | 16.4 | 77.6 | 62.0 | 18.3 | 18.7 | 25.5 | 26.4 |
| Threonine | 16.2 | 21.8 | 16.3 | 44.3 | 45.0 | 26.5 | 16.7 | 19.8 | 27.5 |
| Tryptophan | 5.4 | | | | | 5.6 | 6.3 | 6.6 | 6.7 |
| Valine | 28.2 | 34.5 | 25.0 | 67.9 | 67.0 | 28.7 | 28.8 | 21.7 | 32.7 |
| Dispensable amino acids | | | | | | | | | |
| Alanine | 27.8 | 43.7 | 25.6 | 82.1 | 62.0 | | 26.6 | 21.6 | 41.9 |
| Aspartic acid | 36.9 | 48.8 | 38.7 | 73.0 | 103.0 | | 35.6 | 55.0 | 57.7 |
| Cysteine | 2.2 | 0.2 | 3.5 | 7.6 | 5.0 | 4.2 | 3.2 | 7.7 | 6.5 |
| Glycine | 25.2 | 30.3 | 24.6 | 61.5 | 54.0 | 26.8 | 24.8 | 21.3 | 50.3 |
| Glutamic acid | 45.8 | 63.7 | 46.1 | 131.0 | 102.0 | | 38.4 | 88.6 | 84.1 |
| Proline | 25.1 | 32.7 | 23.6 | 66.8 | 62.0 | | 23.1 | 27.4 | 30.8 |
| Serine | 15.9 | 26.8 | 17.6 | 48.8 | 41.0 | 19.2 | 15.2 | 24.1 | 25.9 |
| Tyrosine | | 34.1 | | 67.1 | 60.0 | 26.5 | 26.9 | 15.5 | 20.1 |

CSBM = conventional soybean meal, DF = defatted, FM = fish meal, FF = full fat.

### 2.2.3 Fatty Acid Profile

The fatty acid content of BSF is shown in Table 2.3. The most abundant saturated fatty acids (SFA) are lauric acid (C12:0), which ranges from 75 to 575. An amount of 6 g/kg, myristic acid (C14:0), which ranges from 23 to 98.7 g/kg, palmitic acid (C16:0), which ranges from 10.3 to 192.0 g/kg, and stearic acid (C18:0), which ranges from 9.8 to 69.0 g/kg. The highest content of monounsaturated fatty acids is oleic acid (C18:1 c9), which ranges from 79.7 to 266.0 g/kg, palmitoleic acid (C16:1), which ranges from 10.3 to 192.0 g/kg, linoleic acid (C18:2n6), which ranges from 38.0 to 314.0 g/kg, and linolenic acid (C18:3n3), which ranges from 9.8 to 36.0 g/kg. SFA content ranges from 362.0 to 782.9 g/kg, MUFA ranges from 85.5 to 287.0 g/kg, n-6 PUFA ranges from 80.0 to 314.0 g/kg and n-3 PUFA ranges from 9.8 to 36.0 g/kg. The MUFA/SFA ratio ranges from 15.3% to 79.3% and the n-3 PUFA/n-6 PUFA ratio ranges from 8.5% to 17.9%.

**Table 2.3** Fatty acid composition of BSF (g kg-1 dry matter basis).

| Type | FF | FF | FF | FF | FF | FF |
|---|---|---|---|---|---|---|
| C10:0 | 20.3 | | 8.6 | 14.3 | | 8.6 |
| C12:0 | 575.6 | 75.0 | 459.7 | 526 | 468.6 | 407.9 |
| C14:0 | 71.4 | 23.0 | 87 | 85.4 | 98.7 | 65.6 |
| C15:0 | | | 1.5 | | 143.8 | 1.3 |
| C16:0 | 10.3 | 192.0 | 122.1 | 109 | 143.8 | 162.7 |
| C18:0 | 9.8 | 69.0 | 25.3 | 15.3 | 17.9 | 14.3 |
| SFA | 782.9 | 362.0 | 707.2 | 750.0 | 742.4 | 664.2 |
| C16:1 | 33.4 | 8.0 | 19.1 | 19.8 | 27.8 | 23.6 |
| c9C18:1 | 79.7 | 266.0 | 112.4 | 61.6 | 77.3 | 182.4 |
| c11C18:1 | 1.2 | | | 2.4 | | |
| MUFA | 119.9 | 287.0 | 134.1 | 85.5 | 115.8 | 218.8 |
| C18:2n-6 | 78.3 | 314.0 | 38.0 | 116.0 | 127.7 | 100.7 |
| n-6 PUFA | 80.0 | 314.0 | 142.2 | 119.0 | 106.0 | 100.9 |
| C18:3n-3 | 11.0 | 36.0 | 16.5 | 10.1 | 9.8 | 16.0 |
| C18:4n-3 | 0.5 | | | | | |
| C20:5n-3 | 2.3 | | | | | 0.2 |
| C22:6n-3 | 0.1 | | | | | |
| n-3 PUFA | 14.3 | 36.0 | 16.5 | 10.1 | 9.8 | 16.2 |
| MUFA /SFA, % | 15.3 | 79.3 | 18.9 | 11.4 | 15.6 | 32.9 |
| n-3 PUFA/ n-6 PUFA, % | 17.9 | 11.5 | 11.6 | 8.5 | 9.0 | 16.1 |

DF = defatted, FF = full fat, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acid.

### 2.2.4 Minerals Composition

Table 2.4 shows the mineral content of BSF. BSF is rich in minerals; calcium (Ca) is the most abundant and ranges from 1.2 g/kg to 35.7 g/kg. Copper (Cu) ranges from 0.1 g/kg to 15.0 g/kg. Iron (Fe) ranges from 0.1 g/kg to 191.0 g/kg. Magnesium (Mg) ranges from 1.0 g/kg to 3.5 g/kg. Manganese (Mn) ranges from 0.2 g/kg to 166.0 g/kg. Phosphorus (P) ranges from 1.0 g/kg to 10.3 g/kg. Potassium (K) ranges from 1.7 g/kg to 15.4 g/kg. Sodium (Na) ranges from 0.7 g/kg to 15.6 g/kg. Zinc (Zn) ranges from 0.7 g/kg to 103.0 g/kg. However, in addition to the accumulation of the above minerals, some toxic and harmful elements (such as Ba, Hg, and Mo) will also bioaccumulate in BSF (Bulak et al., 2020), which will pose a challenge to the safety of feed and food production (Petlum et al., 2019).

**Table 2.4** Mineral compositions of BSF (g kg-1dry matter basis).

| **Type** | **FF** | **DF** | **FF** | **FF** | **FF** |
|---|---|---|---|---|---|
| Calcium (Ca) | 1.2 | 13.0 | 1.9.0 | 34.6 | 35.7 |
| Copper (Cu) | 0.1 | 15.0 | 0.6 | 10.7 | 0.7 |
| Iron (Fe) | 0.1 | 125.0 | 2.1 | 191.0 | 14.0 |
| Magnesium (Mg) | 2.1 | 3.0 | 1.0 | 3.5 | 3.4 |
| Manganese (Mn) | 0.2 | 45.0 | 0.3 | 166.0 | 33.5 |
| Phosphorus (P) | 4.1 | 8.0 | 1.0 | 10.3 | 7.0 |
| Potassium (K) | 6.0 | 11.0 | 1.7 | 15.4 | 9.2 |
| Sodium (Na) | 0.7 | 5.0 | 3.3 | 1.7 | 15.6 |
| Zinc (Zn) | 0.7 | 90.0 | 0.9 | 103.0 | 9.0 |

DF = defatted, FF = full fat.

### 2.2.5 Different Factors of Nutritional Value of BSF

The content of minerals and other nutrients in BSF significantly varies across different studies, and the reasons may be as follows:

First, it may be that the growth stages of BSF are different. On the 4–14th days, the crude fat content of larvae increased rapidly, and the highest level reached 28.4%, while crude protein showed a continuous downward trend at the same developmental stage. With the development of pupa, crude fat dropped

sharply to 24.2%. The maximum crude protein in adulthood is 57.6% and the fat level is 21.6% (Liu et al., 2017).

Second, in relation to the nutritional structure ingested by BSF, the content of fat and ash fed from vegetable waste, chicken feed, and kitchen waste varies greatly (Spranghers Ottoboni et al., 2017; Tschirner and Simon, 2015). In addition, BSF on cow dung grows at a much slower rate of individual size than on poultry feed (Diener et al., 2009; Myers et al., 2014).

Third, it may be related to the processing method. Different killing methods (such as blanching, drying, freezing, high hydrostatic pressure grinding, and asphyxiation) also had an effect on pH, ash, fat content, and oxidative capacity (Larouche et al., 2019; Purba and Paengkoum, 2019). The temperature and method during storage also affect the nutritional quality of BSF (Saucier et al., 2022). Different extraction methods also have different nutrient content; for example, the best separation of protein is through alkali extraction (Caligiani et al., 2018). When the processing temperature is 25℃, the shelf life of BSF can reach seven months (Kamau et al., 2018).

Fourth, it is related to various factors such as temperature, humidity, sunlight, moisture content, pH, etc. Humidity and temperature will obviously affect the incubation, development, and lifespan of BSF (Chia et al., 2018; Tomberlin et al., 2009). Temperatures between 26℃ and 40℃ and relative humidity between 40%–70% are the ideal living conditions for BSF (Holmes, 2010; Sheppard et al., 2002). Sunlight also affects the nutrient composition of BSF, with black soldier flies developing best in the wavelength range between 450 and 700 nm (Park, 2016; Zhang et al., 2010). When the water content in the feed matrix is 60%–80%, the survival rate and growth rate of BSF are the highest (Banks et al., 2014; Cheng et al., 2017). The growth of black soldier flies is better under alkaline conditions than under acidic conditions, and a suitable pH value is between 6– 9 (Ma et al., 2018; Meneguz Gasco et al., 2018).

## 2.3 Antioxidant mechanism of BSF

The BSF has been confirmed to possess antioxidant and immunomodulatory functions, primarily through three mechanisms: chitin, minerals (such as Se and Mg), and lauric acid (C12:0). Chitin or chitosan (β-(1⟶4)-*N-acetyl*-d-glucosamine) is the

second most abundant natural polymer after cellulose and was first discovered in 1884 (Figure 2.1). Under alkaline conditions, chitin structures are prone to alterations, and when these alterations reach 50%, it is referred to as chitosan (Younes and Rinaudo, 2015). Chitin exists in an orderly crystalline microfibril form and is a structural component of the exoskeleton of arthropods or the cell walls of fungi and yeast (Rinaudo, 2013). In general, chitin exhibits its functionality only when the pH is below 6.5 (Synowiecki and Al-Khateeb, 2003). Chitosan and its derivatives can act as antioxidants by scavenging reactive oxygen species (such as hydroxyl, superoxide, and alkyl radicals), or by serving as hydrogen donors to prevent oxidation cascades, and they exhibit highly stable DPPH radicals (Park et al., 2003). Although the exact mechanism of free radical scavenging activity is not fully understood, it is attributed to the reaction of amino and hydroxyl groups (attached to the C-2, C-3, and C-6 positions of the pyranose ring) with unstable radicals, thereby promoting the formation of stable macromolecular radicals (Je et al., 2004).

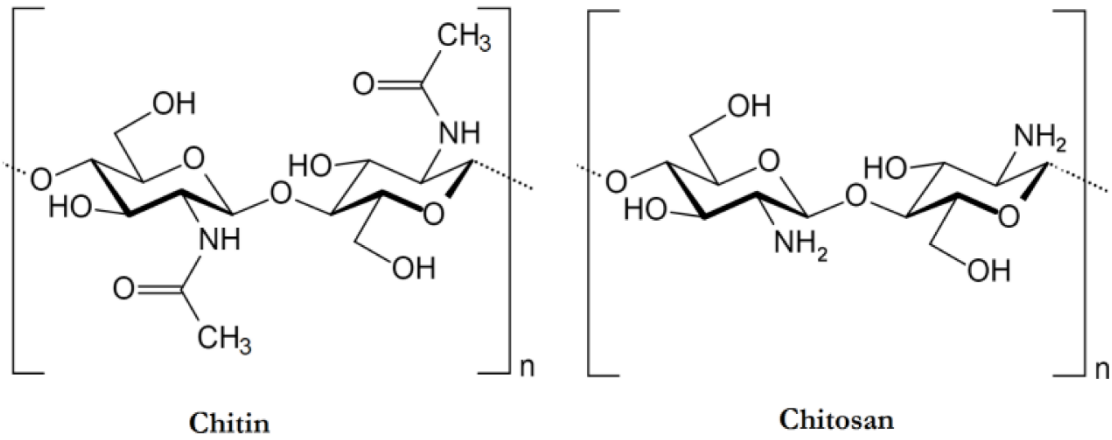


**Figure 2.1** The structure of chitin.

Selenium primarily exerts its antioxidant function in the form of amino acids (selenomethionine and selenocysteine) and enzymes (such as GPX) (Kryukov et al., 2003). Additionally, selenium can regulate the expression of TOAX, GPX, CAT, SOD, and GSH genes to alleviate oxidative stress (Chan et al., 2016). The antioxidant properties of selenium proteins are summarized in Table 2.5.

**Table 2.5** The antioxidant properties of selenium proteins (Xiao et al., 2021).

| **Selenoproteins** | **Function** |
|---|---|
| Selenophosphate synthetase 2 | Selenophosphate synthetase 2 plays a crucial role in the biosynthesis of all selenoproteins, including itself. |
| Selenoprotein W | Antioxidant effects are important for muscle growth. |
| Selenoprotein T | Deficiency leads to early embryonic lethality. |
| Selenoprotein O | Mitochondrial protein consisted of a cytosine-nucleotide-nucleotide-uridine motif suggestive of the redox role. |
| Selenoprotein H | Responsible for Nuclear localization, which is associated with redox sensing and transcription. |
| GPX 1 | Cellular reduction of H2O2. |
| GPX 2 | Reduction of peroxide in the gut. |
| GPX 3 | Reduction of peroxide in the blood. |
| GPX 4 | Causes the Reduction of hydrogen peroxide radicals and facilitates lipid peroxides to water and lipid alcohols and the cellular ferroptosis induced by iron. |

GPX 1 = glutathione peroxidase 1, GPX 2 = glutathione peroxidase 2, GPX 3 = glutathione peroxidase 3, GPX 4 = glutathione peroxidase 4.

BSF is rich in C12:0, which is a medium-chain saturated fatty acid that has been proven to possess antioxidant properties. Research revealed (Nevin and Rajamohan, 2006) that mice fed a diet containing C12:0 showed increased activity of enzymatic antioxidants such as catalase and superoxide dismutase in serum. In addition to enhancing antioxidant enzyme activity, C12:0 also elevated the levels of intracellular reduced glutathione in cell cultures and animal models, actively participating in phase II detoxification systems (Illam et al., 2017).

## 2.4 Effect mechanism of medium-chain fatty acids (MCFA) on rumen

$CH_4$ emissions decrease with the supplementation of dietary MCFA. This can be attributed to the direct inhibition of methane production by MCFA, as well as the reduction in the quantity and activity of rumen protozoa and/or archaea populations (Patra et al., 2017). Consequently, the digestion rates of rumen microbial populations, OM, and NDF associated with fiber substrates also decrease (Kim et al., 2014). Because fatty acids can adsorb onto microbes or feed particles in the rumen, their small molecules readily dissolve in the lipid layer of cell membranes, effectively causing physical damage to cell membranes, disrupting energy metabolism and nutrient transport, leading to the death of cellulolytic bacteria and ciliated protozoa (Kang et al., 2016; Patra, 2009). MCFA reduces the number of rumen microbes, and the intensity of this antimicrobial effect is proportional to the concentration of MCFA (Debruyne et al., 2018). This leads to a decrease in the growth of protozoa, methanogens, and total archaea species. Consequently, the remaining $H_2$ can be optimized through non-inhibited microbial populations, forming $C_3$ through interspecies $H_2$ transfer, reducing the proportion of C2/C3 and $CH_4$ synthesis (Patra, 2016; Wang et al., 2017).

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