


Review

The Potential of Locally-Sourced European Protein Sources for Organic Monogastric Production: A Review of Forage Crop Extracts, Seaweed, Starfish, Mussel, and Insects

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Abstract: Organic monogastric agriculture is challenged because of a limited availability of regional and organic protein-rich ingredients to fulfill the amino acid requirements. The development of novel feed ingredients is therefore essential. The use of starfish (*Asterias rubens*), mussel (*Mytilus edulis*), insect, green and brown seaweed, and forage crop extracts exhibits different approaches to increase protein availability in a sustainable manner through improving the protein quality of existing ingredients, better use of under- or unutilized material, or development of circular bioeconomy. This review assessed limitations and opportunities of producing, processing, and using these novel ingredients in feed. The use of non-renewable resources and the effect on the environment of production and processing the feed ingredients are described. Protein concentration and amino acid quality of the feed ingredients are evaluated to understand their substitution potential compared with protein-rich soya bean and fishmeal. Feedstuffs' effect on digestibility and animal performance is summarized. With the exception of seaweed, all novel ingredients show potential to partly substitute fishmeal or soya bean fulfilling part of the protein requirement in organic monogastric production. However, improvements during production and processing can be made to enhance protein quality, sustainability of the novel ingredients, and nutrient utilization of novel feed ingredients.

Keywords: novel feed ingredient; sustainability; pig; poultry; protein; amino acid; digestibility; growth performance



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1. Introduction

In the European Union, at least 30% of protein feed for organic monogastrics must be made up of regionally-grown ingredients. Moreover, after 2025, all of the feed for organically produced monogastrics has to come from organic feed sources of agricultural origin within a year of feeding [1]. A limited availability of high quality and regionally produced protein sources certified for organic monogastric production hinders the sufficient supply of organic protein and thus amino acids (AA) to monogastrics' diets. In organic animal husbandry, the protein quality of feed ingredients is of particular importance since animal requirements for essential AA (EAA) have to be fulfilled without the addition of crystalline AA [2]. The use and development of alternative feed ingredients is therefore essential.

Alternative feed ingredients are typically evaluated in terms of their potential to substitute the conventional ingredients, fishmeal, or soya bean. This is due to their high protein level and the good AA profile, i.e., fitting animal's requirements. Nonetheless, it is desirable to lower the dependency on soya and fishmeal as both of these conventional feed ingredients can have negative effects on environmental and economic sustainability. Soybean meal causes deforestation and emissions from long transport distances [3].

Although organic deforestation-free soya can be used, Northern European countries, in particular, still depend on imports because production within Europe largely takes place in Eastern and Southern Europe [4]. Overfishing negatively affects ecosystem resilience [5]. Variation in availability due to, e.g., overfishing [6] or limited agricultural land area as well as competition for these ingredients leads to high and volatile prices. Competition for these resources may increase further with the predicted increase in human population growth since this is expected to enhance demand for protein-rich agricultural commodities [7]. Therefore, protein sources that do not compete with human food are desirable.

Blue mussel (*Mytilus edulis*), starfish (*Asterias rubens*), seaweed, protein extracted from forage crops (green protein) and insects can all be produced in Northern Europe. Moreover, all of these ingredients can play a role in circular and sustainable feed production. Although red seaweeds contain a high protein fraction, the focus in this review will be on three seaweeds that are common and abundant in Northern Europe and have been of interest for animal feed: *Ascophyllum nodosum*, *Saccharina latissima* and *Ulva lactuca*. In terms of insects, focus will be on the black soldier fly (*Hermetia illucens*), the yellow mealworm (*Tenebrio molitor*), and the lesser mealworm (*Alphitobius diaperinus*). Production of these insects is already established and there is potential for them to be produced at large scale [8]. However, processed insects are not yet allowed in monogastrics' feed under EU regulation. Living insects are allowed in poultry nutrition. Protein extracts from perennial ryegrass (*Lolium perenne*), lucerne (*Medicago sativa* L.), and red clover (*Trifolium pratense* L.) are included in the review as these forage crops are commonly grown in Northern Europe either as monocultures or as constituents of forage mixtures.

In this review, the potential of using cultivated blue mussel, starfish, seaweed, protein extracted from forage crops (green protein), and insects as alternative feed ingredients in European organic monogastric nutrition will be evaluated on the basis of their potential sustainability, nutrient composition, and their effect on animal performance.

2. Sustainability

2.1. Sustainability during Production

EU rules for organic production promote sustainable agriculture and encompass the efficient and restricted use of external inputs and non-renewable resources ([1]; articles 5 and 6). The growth or cultivation of aquatic ingredients, i.e., seaweed, starfish, and mussel, requires no arable land because they grow in water. Although wild harvest is the main harvesting method of seaweeds in Europe [9], interest in seaweed cultivation is increasing [10,11]. Starfish are a by-product of mussel cultivation in which overabundant starfish are caught to prevent losses in mussel populations due to predation. The rearing of insects does not require a low amount of non-agricultural land, moreover, insect feed can be associated with the use of arable land and water. When cultivating perennial forage crops from which protein can be extracted, arable land is used in a more sustainable way compared with annual crops such as seed crops. Perennial crops cover the soil all year round and have longer growing seasons [12,13]. Moreover, their extensive root systems increase competitiveness against weeds and biomass yields [12,13].

Growing aquatic ingredients does not require any non-renewable resources, such as N, P, or water, during growth because they utilize nutrients that naturally occur in the water. Mussels effectively remove 0.60–1.27 t N ha⁻¹ and 0.04–0.10 t P ha⁻¹ from the water [14]. The removal of these nutrients from the water promotes circular production by mitigating eutrophication in areas with an excess of nutrients [15]. Similarly, the removal of starfish and seaweed will give a net removal of nutrients from the water, although to a lesser extent than mussels. Seaweeds are less efficient biofilters than mussels [16]. Conversely, insects and forage crops need external input of non-renewable resources for growth. For forage crops, the nutrient input depends on the cultivation method. Forage crops are effective at capturing nutrients and water and consequently minimize nutrient leaching [17] and increase soil N supply in crop rotations [18,19]. For insects, the input of resources depends on the utilized feeding substrate and insects' feed efficiency. Insects

can be highly efficient in turning low-quality biomass into high-quality protein, but this largely depends on diet composition as well as insect species [20,21]. The high efficiency is related to the fact that insects are poikilothermic, i.e., they do not use energy for regulation of body temperature. This enables them to utilize ingested energy for metabolic processes instead of thermoregulation. The diet for insects should consist of previously unutilized biomass such as waste and low-value side streams, and not traditional livestock feedstuffs, to be truly sustainable. However, currently, there are limitations for the feed allowed in insect rearing. Since insects are considered livestock, the ingredients allowed in insects' diets are subjected to EU regulation [22], which lays down restrictions on the use of among others catering waste and processed animal protein. Moreover, in the future additional restrictions may apply for organically reared insects under EU legislation.

Knowledge on energy use and potential greenhouse gas (GHG) emissions is relevant in organic agriculture to comply with responsible use of energy and low-carbon economy ([1]; article 5). Anthropogenic GHG emissions and utilization of external energy are non-existent during the growing period of the aquatic ingredients. In fact, seaweeds may be a sink for carbon sequestration [23]. Similarly, some perennial forage crops and legumes can sequester carbon in soils. Moreover, legumes contribute to reduced GHG emissions because of decreased GHG release and reduced fossil energy input [24]. Energy is, however, required to regulate temperature during the production of poikilothermic insects. Oonincx and De Boer [25] found that 43% of energy used during insect production was due to the use of heat and electricity. Furthermore, GHG release may be associated with metabolic processes and microbial respiration during insect production [26]. Parodi et al. [26] observed that carbon was mainly emitted as CO₂. The GHG emissions may also be associated with the feed used for insect rearing. In order to fully understand the environmental sustainability of insects as an alternative to soya bean, the global warming potential of insects compared to soya bean should be established. Besides emissions directly linked to the production process, CO₂ emissions are often related to transport. Therefore, EU organic regulation stimulates short distribution channels and local production ([1]; article 4). Mussel, seaweed, and starfish species discussed in this review all occur in Northern Europe, hence in many coastal areas they could fulfil part of the requirements for locally-sourced feed in organic monogastric production. Regional production is also possible for insects, which are not bound to land, and forage crops.

EU regulation requires stimulation of biodiversity and no harm to the environment ([1]; articles 4 and 5). Relative to annual crops, cultivation of perennial crops has substantial ecological and economic benefits because of reduced soil erosion and a provision of a continuous habitat for wildlife [17]. The quantity and harvest method of seaweeds, starfish and mussel may affect the surrounding fauna as well as population dynamics and should therefore be taken into account when determining the method and quantity to be harvested. In this regard, the harvest of starfish did not destroy the underlying sea bed when using a purse seine [27]. Moreover, this method had low by-catch and did not affect biodiversity of infauna or abundance of species in the Danish Limfjord. The impact of mussel cultivation depends on the cultivation method. Dredging of the bottom when mussels are grown on natural beds ruins the seabed, and therefore methods using line or net cultivation are more sustainable [28]. Furthermore, mussels have been found to improve water transparency and reduce chlorophyll *a*, which is related to the presence of plankton, bacteria, larvae, fish eggs, and other small biological organisms, in a large area surrounding a mussel cultivation site [29]. Furthermore, even though excretion of nutrients by mussels will increase local sedimentation of nutrients, nutrient loading is reduced when taking into account an entire water body due to net removal of nutrients [29].

The harvested quantity of different ingredients affects their availability for feed. A consequence of extensive production of aquatic products is fluctuation in quantity depending on biological factors. Low quantities, i.e., quantities that do not sustain profitability of harvest and processing, can be a bottleneck for the continuous production of aquatic ingredients for feed. In contrast, perennial crops for green protein and insects are grown

with the help of human interventions, hence quantities are less variable. Insect production can be scaled under controlled circumstances according to demand and feed substrate availability. The quantity of perennial crops is affected by available arable land mass, which can also be used for monocultures of annual crops, which are sown on more than two-thirds of global cropland for animal feed production, human consumption, and non-food goods (e.g., biofuel) [12,17].

Availability for feed is also dependent on the competition for these ingredients as food as well as non-food goods. Competition for forage crops can be large because of its multiple purposes. Traditionally, forage crops are used for animal production, mostly for ruminants; however, recently the crops have been considered for production of monogastric feed protein as well as for non-nutrition purposes such as production chemicals and materials [30,31]. Insects are mainly of interest for their nutritious quality as a feed or food. However, the use of insects for food is limited by consumer perception of insects as food [32]. In contrast, starfish and mussels intended for feed do not compete with food. This is because mussels intended for feed are either waste mussels, i.e., undersized mussels [33], or mussels farmed to mitigate water eutrophication, which are generally less suited for human consumption because of uneven and small sizes [29]. Besides quantity, also quality of the products should be taken into account as this determines how efficiently feed ingredients can be converted into animal protein. This will be reviewed in upcoming sections.

2.2. Sustainability at Processing

The processing of the ingredients studied in this review requires low input of external processing aids and can be done using mechanical and physical processes. Responsible use of energy should be taken into account for the organic processing of feed ingredients. Heat, and thus energy is required for drying and grinding, which is essential to prevent microbial spoilage and to elongate shelf life [34,35]. The dry matter (DM) of seaweed can be as low as 14% and thus requires a significant effort to dry [36]. Energy used for processing of seaweeds is for up to 60% taken up by drying [37].

High input of energy, as well as water, is also required when processing mussels because they are generally boiled to remove the shell and to obtain the protein-rich meat fraction. It is difficult to develop alternative processing methods that provide a meal of equally high quality. Alternative methods will often have parts of the shell remaining in the meal fraction. This means that the contribution of protein will be lower, whereas the contribution of ash will be higher, because mussel shell is made up of around 94% ash in DM [38].

Pre-processing can also be done for insects. The black soldier fly is often defatted mechanically, as this insect can have a fat content of close to 40% [39,40]. A benefit of defatting is the generation of a product with a higher protein concentration.

The improvement of product quality is also the main merit of protein extraction from forage crops. The efficient use of green biomass (annual and perennial) as a source of monogastric feed protein requires separation of the protein from the plant fiber matrix. Numerous different separation techniques have been applied; however, several recent studies have applied screw-press processing to achieve a protein-rich juice and a fibrous pulp [41–45]. Soluble proteins in the plant juice can be extracted via different methods where heat treatment, acidification, or fermentation are commonly used methods [46]. The result is a protein extract or concentrate commonly referred to as green protein and a residual brown juice [44]. Efficient utilization of the produced fractions is a major contributor to the sustainability of the biorefining concept [47]. Production of a protein concentrate for monogastrics as a sole product will limit the competitiveness of the production, and the biorefining concept must include efficient use of the pulp fraction [41,48]. The pulp fraction may be used as feed for ruminants [49], for biogas [50] or bioethanol production [35]. The methods applied all require fresh biomass due to the need for intact protein for precipitation [43]. This requirement introduces seasonal challenges with biomass supply and

hence limits the running hours on the biorefinery. Using ensiled forage crops as input for the biorefinery and a subsequent feeding with the plant juice is tested as alternative approaches [51], which would make production during the winter possible and would reduce the need for drying.

3. Nutrient Composition of Alternative Ingredients

In this section, an overview of the nutrient composition of meal from mussel, starfish, seaweed, insects, and forage crop protein extract will be given from data extracted from literature on either dried or fresh ingredients. Units of extracted data have been equalized, and average, standard deviation (SD), and coefficient of variation (CV) have been calculated. Nutrient levels, in particular crude protein (CP, N \times 6.25) and EAA, in alternative feed ingredients will be compared to those in fishmeal or organic soya bean in order to determine their substitution potential based on nutritional value. The chemical composition of the aforementioned conventional ingredients is depicted in Table 1. Table 1 also contains average data for wheat (non-organic), which is more similar to seaweed in terms of CP concentration. Besides the nutrient concentration, also the variation in nutrient composition of the different feed ingredients among studies will shortly be discussed because harvesting and growing conditions affect nutrient composition of alternative feed ingredients. In the different tables, the coefficient of variation and SD are used to visualize the nutrient variability within feed ingredients among different studies.

Table 1. Chemical composition of macronutrients (g/100 g DM), minerals (g/kg DM) and amino acid (AA) profile (g/100 g CP) in meal from commonly fed ingredients.

Item	Wheat ¹			Fish Meal ²			Organic Soya Bean ³		
	N	Avg \pm SD ⁴	CV ⁵	N	Avg \pm SD	CV	N	Avg \pm SD	CV
Crude ash	6	1.83 \pm 0.18	10.0	5	18.8 \pm 2.66	14.2	3	6.41 \pm 0.44	6.91
Crude fat	7	2.15 \pm 0.42	19.5	4	9.51 \pm 0.47	4.97	3	14.5 \pm 6.86	47.3
Crude protein	8	14.2 \pm 2.00	14.1	6	71.4 \pm 2.45	3.43	5	44.6 \pm 3.65	8.17
<i>Minerals</i>									
Calcium	6	0.43 \pm 0.27	62.4	4	41.4 \pm 8.52	20.6	2	3.55 \pm 0.15	4.23
Phosphorus	5	4.02 \pm 0.48	12.0	4	30.4 \pm 6.15	20.2	2	6.15 \pm 0.75	12.2
<i>EAA ⁶</i>									
Histidine	4	2.58 \pm 0.24	9.17	5	2.52 \pm 0.58	23.1	5	2.65 \pm 0.08	2.97
Isoleucine	8	3.36 \pm 0.19	5.53	5	4.00 \pm 0.11	2.79	5	4.54 \pm 0.24	5.36
Leucine	4	6.47 \pm 0.17	2.59	5	6.88 \pm 0.31	4.57	5	7.60 \pm 0.18	2.42
Lysine	8	3.18 \pm 0.30	9.56	5	7.47 \pm 0.37	4.99	5	6.09 \pm 0.16	2.69
Methionine	8	1.53 \pm 0.08	5.06	5	2.68 \pm 0.12	4.63	5	1.40 \pm 0.06	3.93
Phenylalanine	4	4.30 \pm 0.23	5.33	5	3.83 \pm 0.17	4.51	5	4.95 \pm 0.19	3.86
Threonine	8	2.90 \pm 0.11	3.76	5	4.01 \pm 0.23	5.80	5	3.86 \pm 0.20	5.10
Tryptophan	5	1.17 \pm 0.05	3.98	2	0.93 \pm 0.08	8.78	4	1.36 \pm 0.18	13.1
Valine	5	4.32 \pm 0.26	5.97	5	4.66 \pm 0.12	2.67	5	4.84 \pm 0.24	5.02
<i>NEAA ⁷</i>									
Alanine	8	3.36 \pm 0.40	11.8	5	6.17 \pm 0.10	1.68	5	4.24 \pm 0.20	4.72
Arginine	5	4.97 \pm 0.40	8.00	5	5.75 \pm 0.24	4.20	5	7.38 \pm 0.32	4.28
Aspartic acid	4	5.37 \pm 0.38	7.13	5	6.93 \pm 3.24	46.7	5	11.3 \pm 0.55	4.86
Cysteine	4	2.20 \pm 0.16	7.23	5	1.11 \pm 0.64	57.8	5	1.54 \pm 0.12	7.80
Glutamic acid	4	26.9 \pm 2.22	8.26	5	12.2 \pm 1.08	8.83	5	18.0 \pm 0.48	2.65
Glycine	4	4.28 \pm 0.32	7.48	5	6.99 \pm 0.60	8.63	5	4.20 \pm 0.14	3.45
Proline	4	8.19 \pm 1.50	18.4	5	4.91 \pm 0.64	13.0	5	4.53 \pm 1.18	26.0
Serine	4	4.50 \pm 0.34	7.49	5	4.04 \pm 0.85	21.1	5	4.86 \pm 0.41	8.39
Tyrosine	2	2.97 \pm 0.12	4.17	5	3.69 \pm 1.11	30.1	4	3.39 \pm 0.13	3.71
Σ AA	4	90.6		5	88.2		5	95.7	
Σ EAA	4	29.1		5	36.4		5	37.0	
Σ NEAA	4	61.5		5	51.8		5	58.7	
EAA:NEAA	4	0.47		5	0.70		5	0.63	

¹ Based on literature values: [52–57]; ² Based on literature values [58–63]; ³ Based on literature values: [64–67]; ⁴ Average \pm standard deviation over included studies; ⁵ Coefficient of variation over included studies; ⁶ Essential amino acids; ⁷ Non-essential amino acids.

3.1. Mussel Meal and Starfish Meal as Compared to Fishmeal

Mussel meal (*Mytilus edulis*) and starfish meal (*Asterias rubens*; Table 2) are both animal protein and therefore a comparison with fishmeal will be made. Mussel meal contains a high level of CP at 66% in dry matter (DM), which is slightly below the 71% CP in DM in fishmeal. Starfish meal, on the other hand, has a considerably lower CP content (39% of DM) than fishmeal. On the basis of CP concentration, mussel meal will contribute a similar amount of CP to the complete diets as fish meal, hence mussel meal could almost completely substitute fishmeal. Starfish meal, however, will contribute less CP, and conversely less AA to the complete feed. The ratio of EAA to non-essential (NEAA) is similar between fishmeal and mussel meal, whereas starfish meal has a lower ratio than either of these feed ingredients. This coincides with lower levels of most of the EAA (g/100 g CP) in starfish meal compared with fishmeal or mussel meal. The EAA profile is comparable for all three feed ingredients in that lysine and leucine are most abundant, and tryptophan and histidine are least abundant. A high content of EAA, particularly lysine and leucine, fits with pigs' high requirements for these AA [68]. The lower methionine concentration in starfish meal compared with mussel meal and fishmeal makes it a less suitable source of methionine. The high ash concentration in starfish meal (35–55% DM) is the main limiting factor for its inclusion in complete diets because of a wide Ca:P ratio.

The fat content of mussel meal and starfish meal is more variable than that in fishmeal. Moreover, the protein content of starfish meal is highly variable in comparison with fishmeal and mussel meal. Changes in the environmental conditions of mussel and starfish will alter growth conditions and nutrient availability. Environmental factors, such as salinity, temperature, light intensity, food availability and oxygen saturation, vary depending on the season [33,69–71]. A main determining factor for nutrient variation of starfish and mussel is the reproductive cycle [33,72] which alters energy and protein requirements. Seasonal variation on CP, fat, and ash content of starfish was indeed related to the reproductive cycle in a study on annual variation of macronutrients of starfish from Danish waters in a one-year study [73]. On the basis of an inverse relation between CP and ash, an optimum harvesting time of starfish with low ash and high CP level could be determined. However, it is not known how annual and spatial differences affect the optimum harvesting time of starfish. Mussel meal has a less variable concentration of ash and protein than starfish meal. This might be related to the cultivation method; mussels, produced for environmental mitigation purposes, are grown under controlled conditions at established and fixed production sites. Starfish, on the other hand, are not produced under controlled conditions because they are harvested from the seabed. Therefore, nutrient composition within and between batches of starfish may be more variable although harvesting at the optimum time will likely minimize this variation.

Table 2. Chemical composition of macronutrients (g/100 g DM), minerals (g/kg DM), and amino acid (AA) profile (g/100 g CP) in meal from blue mussel meat (*Mytilus edulis*) and starfish meal (*Asterias rubens*).

Item	Mussel Meal ¹			Starfish Meal ²		
	N	Avg ± SD ³	CV ⁴	N	Avg ± SD	CV
Crude ash	5	8.61 ± 0.91	10.5	15	45.0 ± 9.98	22.2
Crude fat	5	10.1 ± 3.26	32.2	15	8.28 ± 1.40	16.8
Crude protein	5	66.4 ± 3.86	5.82	15	39.0 ± 9.69	24.8
<i>Minerals</i>						
Calcium	2	7.00 ± 2.00	28.6	15	129 ± 31.5	24.5
Phosphorus	2	8.50 ± 0.50	5.88	15	6.31 ± 5.64	89.4
<i>EAA</i> ⁵						
Histidine	5	1.85 ± 0.17	9.32	15	1.65 ± 0.11	6.53
Isoleucine	5	3.96 ± 0.57	14.3	15	3.61 ± 0.12	3.42
Leucine	5	6.10 ± 0.83	13.7	15	5.36 ± 0.35	6.60

Table 2. Cont.

Item	Mussel Meal ¹			Starfish Meal ²		
	N	Avg ± SD ³	CV ⁴	N	Avg ± SD	CV
Lysine	5	6.78 ± 0.69	10.1	15	5.65 ± 0.38	6.78
Methionine	4	2.37 ± 0.40	16.7	15	1.95 ± 0.18	9.15
Phenylalanine	5	3.43 ± 0.45	13.2	15	3.22 ± 0.15	4.52
Threonine	5	4.18 ± 0.51	12.3	15	4.40 ± 0.53	12.0
Tryptophan	1	1.20		14	0.98 ± 0.10	10.6
Valine	5	3.97 ± 0.51	12.8	15	4.49 ± 0.37	8.27
NEAA ⁶						
Alanine	5	4.57 ± 0.41	9.07	15	5.00 ± 0.44	8.73
Arginine	5	6.49 ± 0.77	11.9	15	5.82 ± 0.22	3.82
Aspartic acid	5	9.00 ± 1.16	12.9	15	8.43 ± 0.15	1.76
Cysteine	4	1.51 ± 0.50	32.7	15	1.39 ± 0.22	15.7
Glutamic acid	5	11.8 ± 1.31	11.1	15	10.0 ± 2.39	23.9
Glycine	5	5.65 ± 0.50	8.78	15	14.2 ± 1.97	13.9
Proline	5	3.52 ± 0.42	11.8	15	4.50 ± 0.35	7.75
Serine	5	4.11 ± 0.69	16.8	15	5.08 ± 1.13	22.2
Tyrosine	3	3.29 ± 0.56	17.1	1	2.94	
∑AA	5	80.8		15	85.8	
∑EAA	5	32.4		15	31.2	
∑NEAA	5	48.3		15	54.6	
EAA:NEAA	5	0.67		15	0.58	

¹ Based on literature values: [38,74–77]; ² Based on literature values: [63,73,74,78]; ³ Average ± standard deviation over included studies; ⁴ Coefficient of variation over included studies; ⁵ Essential amino acids; ⁶ Non-essential amino acids.

3.2. Seaweed Meal as Compared to Soya Bean and Wheat

Seaweeds' nutrient composition (Table 3) will be compared to soya bean and to wheat. Brown seaweeds have a low protein concentration of 7.6 and 10.7% CP in DM for *S. latissima* and *A. nodosum*, respectively. The green seaweed, *U. lactuca*, contains about twice as much protein, i.e., 19.0% CP in DM on average. Protein levels of seaweeds are clearly lower than what is found in soya bean (Table 1). Hence, seaweeds will supply little protein to complete diets for monogastric animal and thus, in their intact form, will not be a good substitute for protein-rich feed ingredients. In terms of protein level, they are more suitable as a substitute for wheat (Table 1). Despite the low CP level, on average *A. nodosum* and *U. lactuca* contain a similar, slightly lower, ratio of EAA:NEAA compared to soya bean. This ratio is clearly lower for *S. latissima*, which has a ratio comparable to wheat. Besides supplying less protein, the protein fraction in seaweeds also contains less lysine compared to that in soya bean. This means that more lysine has to come from another source in order to fulfil monogastrics' high requirements for lysine. Leucine is the most abundant AA in all seaweeds. With the exception of *A. nodosum*, leucine levels in seaweeds are comparable to that in soya bean. Histidine and methionine in g/100 g CP are low in all seaweeds. However, the relative methionine levels in seaweeds are similar to the levels in soya bean and wheat. The histidine levels of all seaweeds, on the other hand, are clearly lower than that in soya bean. Carbohydrates make up the majority of seaweeds, i.e., at least 50% of DM. The carbohydrate fraction is made up of mostly indigestible polysaccharides from the algae cell wall [79]. The nutritional value of these carbohydrates depends on their fermentation potential, which provides the animal with some energy from short chain fatty acids. A high inclusion of seaweed meal with indigestible fiber will lead to a dilution of digestible macronutrients in the diet. The high fiber fraction will, moreover, limit the availability of digestible nutrients. Polysaccharides may limit absorption of other nutrients. Firstly, non-digestible polysaccharides can trap protein in cellular matrix, thereby making protein inaccessible for digestive enzymes [80]. Acid detergent lignin is negatively correlated with in vitro protein digestibility of seaweeds [81]. Alginates, which are prevalent in brown seaweeds [82], can increase digesta viscosity and thereby limit the efficient distribution of enzymes [83]. Lastly, phenolic compounds in seaweeds may depress protein digestibility by precipitating dietary protein [84]. Seaweeds contain between 15% and 30% crude ash (Table 2). This is considerably higher than the ash level

in soya bean. Similar to fiber, ash concentration of seaweed limits the inclusion level in feed. Firstly, a too high inclusion of seaweeds will dilute the feed ration thus allowing less space for required nutrients. Secondly, an excess in certain minerals may be toxic and reduce animal performance [68]. Moreover, excess minerals will end up in the environment because mineral concentrations in the body are tightly regulated [85]. Most macro-minerals in seaweeds are present at levels far above those in wheat or soya bean. Except for an excess in minerals, also imbalances among minerals may limit seaweeds' inclusion level in feed rations. The balance among Na, Cl, and K can affect the electrolyte balance in the animal, which affects optimum growth performance [86]. Moreover, low phosphorus, which is present at a lower concentration in seaweeds than in the conventional feed ingredients, will contribute to a wide and undesirable Ca:P ratio.

All nutrients within seaweed species are highly variable among studies. Seaweeds are grown in natural waters, and therefore location, season, and environmental factors, such as pH, temperature, sunlight, and nutrient content of water, affect their nutrient composition [36,87,88]. Therefore, each harvested batch of seaweeds should be analyzed if they are to be used as feed ingredients. The seaweeds described in this review provide only a very low amount of protein; therefore, intact seaweed may not be a good protein source to fulfil the gap in available feed protein. The currently low production potential combined with a high energy input at drying makes it difficult to develop these seaweed into sustainable animal feed for organic monogastric animals. Therefore, intact brown and green seaweeds will not be further discussed in this review.

Table 3. Chemical composition of macronutrients (g/100 g DM), minerals (g/kg DM), and amino acid (AA) profile (g/100 g CP) in green (*U. lactuca*) and brown seaweeds (*A. nodosum* and *S. latissima*).

Item	<i>Ulva lactuca</i> ¹			<i>Ascophyllum nodosum</i> ²			<i>Saccharina latissima</i> ³		
	N	Avg ± SD ⁴	CV ⁵	N	Avg ± SD	CV	N	Avg ± SD	CV
Crude ash	17	19.9 ± 5.28	26.6	11	25.2 ± 2.57	10.2	20	21.5 ± 8.56	39.8
Crude fat	16	2.28 ± 1.87	81.9	7	3.94 ± 2.29	58.2	2	3.15 ± 2.36	74.9
Crude protein	18	19.0 ± 5.94	31.3	14	7.56 ± 2.63	34.7	28	10.7 ± 7.36	69.0
Carbohydrate	8	51.6 ± 7.73	15.0	2	62.9 ± 6.75	10.7	21	53.5 ± 16.4	30.7
<i>Minerals</i>									
Calcium	5	24.5 ± 19.7	80.5	9	10.57 ± 2.41	22.8	7	39.6 ± 38.9	98.3
Magnesium	3	23.0 ± 5.30	23.0	9	8.31 ± 1.68	20.2	7	6.79 ± 0.69	10.2
Phosphorus	3	1.66 ± 0.42	25.2	8	1.96 ± 1.98	101	7	2.99 ± 0.72	23.9
Sodium	4	18.0 ± 7.35	40.9	8	42.89 ± 7.92	18.5	7	46.0 ± 4.93	10.7
Potassium	4	20.2 ± 3.45	17.1	9	33.20 ± 21.3	64.3	7	61.0 ± 20.3	33.3
<i>Micro-minerals</i>									
Aluminum	1	122		4	89.2 ± 69.7	78.1	16	376 ± 513	137
Arsenic	4	5.72 ± 3.36	58.7	4	37.5 ± 11.8	31.4	16	57.6 ± 22.5	39.1
Cadmium	3	0.27 ± 0.27	101	4	0.95 ± 0.58	61.0	8	1.17 ± 0.49	41.5
Chromium	3	2.66 ± 1.19	44.8	4	1.83 ± 0.68	37.4	8	0.61 ± 0.50	82.7
Cobalt	3	1.06 ± 0.57	53.9	4	3.05 ± 1.01	33.2	7	0.16 ± 0.10	65.6
Copper	6	30.0 ± 52.4	174	7	8.76 ± 8.60	98.2	16	2.41 ± 1.09	45.4
Iodine	2	68.7 ± 45.4	66.1	1	461		15	1549 ± 1799	116
Iron	5	899 ± 686	76.3	8	185 ± 88.1	47.6	16	330 ± 394	119
Lead	3	0.85 ± 0.50	59.0	4	0.49 ± 0.01	1.70	8	0.90 ± 1.44	159
Manganese	5	47.4 ± 41.0	86.6	8	19.8 ± 11.4	57.3	16	14.6 ± 12.4	84.8
Nickel	4	5.35 ± 1.85	34.5	4	1.85 ± 0.67	36.0	8	0.76 ± 0.38	50.4
Selenium	2	1.07 ± 0.88	82.2	4	0.98 ± 0.02	1.70	7	1.60 ± 1.38	86.0
Zinc	5	26.5 ± 19.7	74.5	7	58.2 ± 20.3	35.0	16	34.9 ± 17.1	49.2
<i>EAA</i> ⁶									
Histidine	6	1.45 ± 0.24	16.3	3	1.32 ± 0.16	11.8	17	1.51 ± 0.23	14.9
Isoleucine	6	3.49 ± 0.83	23.7	3	2.84 ± 0.86	30.5	17	2.68 ± 1.11	41.5
Leucine	6	6.16 ± 1.27	20.6	3	4.80 ± 1.82	37.8	17	6.27 ± 0.90	14.4
Lysine	6	4.14 ± 1.22	29.4	3	4.35 ± 0.47	10.9	17	4.23 ± 1.17	27.5
Methionine	6	1.69 ± 0.57	33.8	3	1.34 ± 0.65	48.7	17	2.09 ± 0.28	13.4
Phenylalanine	6	3.57 ± 0.78	21.7	3	3.04 ± 1.29	42.3	17	4.36 ± 0.71	16.3

Table 3. Cont.

Item	<i>Ulva lactuca</i> ¹			<i>Ascophyllum nodosum</i> ²			<i>Saccharina latissimi</i> ³		
	N	Avg ± SD ⁴	CV ⁵	N	Avg ± SD	CV	N	Avg ± SD	CV
Threonine	6	4.59 ± 1.08	23.5	3	3.47 ± 1.09	31.3	17	4.57 ± 0.42	9.10
Tryptophan				1	0.14		6	1.74 ± 0.22	12.9
Valine	6	5.79 ± 1.75	30.2	3	3.56 ± 1.12	31.5	17	4.19 ± 0.76	18.2
NEAA ⁷									
Arginine	6	4.89 ± 0.94	19.2	3	3.13 ± 0.99	31.8	17	5.33 ± 1.11	20.9
Alanine	5	1.19 ± 0.60	50.3	4	4.59 ± 3.24	70.6	17	11.6 ± 3.01	25.9
Aspartic acid	6	10.1 ± 2.78	27.6	4	6.42 ± 3.18	49.5	17	15.3 ± 3.89	25.4
Cysteine	5	2.27 ± 1.90	83.5	1	1.80		17	6.82 ± 6.20	90.9
Glutamic acid	6	10.3 ± 1.87	18.1	4	10.1 ± 6.84	67.8	17	17.5 ± 3.81	21.8
Glycine	6	6.07 ± 2.26	37.2	2	4.85 ± 0.05	1.02	17	8.56 ± 2.57	30.0
Proline	6	4.03 ± 0.83	20.6	4	2.66 ± 1.72	64.6	17	4.89 ± 2.08	42.5
Serine	6	4.72 ± 1.37	29.1	3	4.19 ± 0.15	3.46	17	5.93 ± 1.32	22.3
Tyrosine	4	2.96 ± 1.78	60.3	2	1.37 ± 0.50	36.8	16	1.96 ± 1.18	60.2
∑AA	6	83.0		3	66.8		17	108	
∑EAA	6	30.9		3	24.8		17	30.5	
∑NEAA	6	52.1		3	42.1		17	77.8	
EAA:NEAA	6	0.60		3	0.61		17	0.42	

¹ Based on literature values: [89–108]; ² Based on literature values: [84,88,109–117]; ³ Based on literature values: [36,84,87,92,102,103,118,119];

⁴ Average ± standard deviation over included studies; ⁵ Coefficient of variation over included studies; ⁶ Essential amino acids; ⁷ Non-essential amino acids.

3.3. Insect Meal as Compared to Soya Bean

Nutrient composition of insect larvae meals is shown in Table 4. Black soldier fly is depicted in full fat as well as in defatted form. Black soldier fly contains the most ash, and the mineral composition differs from that in yellow and lesser mealworm. Calcium is almost 10-fold more abundant in black soldier fly than in either of the two mealworms. Phosphorus is the next most abundant mineral in black soldier fly and is similar among all insect meals. Therefore, there is a wider Ca:P ratio in black soldier fly compared to the other insect meals. Chitin and ADF levels vary between and within insect species. The acid detergent fiber (ADF) fraction of insects is believed to be representative of the chitin derived from the insect cuticle, i.e., part of the exoskeleton [120]. The level of chitin in insect meal is of importance because it can negatively affect protein digestibility as some AA in insect meal are bound to chitin [120,121]. The protein concentration in insect meal, with the exception of full fat black soldier fly, is higher than that in soya bean. The lower protein concentration in full fat black soldier fly is due to the high fat content. Fat in black soldier fly can reach levels of up to 40% of DM [122]. The variation in crude fat content for defatted black soldier fly is due to varying degrees of processing. Insects have an EAA:NEAA ratio similar to or higher than that observed in soya bean. The most abundant EAA is leucine for all insect types. This is also the most abundant EAA in soya bean. Furthermore, lysine and valine are highly abundant in insect meals. The range of most EAA in insect meals is similar to values observed for EAA in soya bean. Phenylalanine, however, is lower in insect meals than in soya bean. The ratio between phenylalanine and lysine of the insects (0.59–0.74:1) is equal or above the established ideal ratio to meet minimum requirements for pigs [68,123] and should therefore not restrict feed formulation. Finally, several studies find low values of cysteine, i.e., below 1 g/100 g CP, in the different insect meals. Sufficient levels of cysteine are important in order to balance methionine and cysteine in diet formulations. Sulphur-containing amino acids are often difficult to supply at adequate levels in organic diet formulations [124]. Hence, the quality of insect meal may be variable depending on the level of cysteine and methionine. The variation within AAs is stronger for all insect meals than for soya bean. Rearing substrate may be one important determinant for insects' nutrient composition [20,125]. Knowledge on variation in AA composition and chitin bound N will be particularly useful as this determines protein quality. It will be useful to better understand factors at rearing and processing that affect the nutrient composition and protein quality of insect meals when using them in animal

feed, and, as for most other new feed ingredients, an analysis of nutrient composition will be valuable before diets are composed.

Table 4. Chemical composition of macronutrients (g/100 g DM), minerals (g/kg DM), and amino acid (AA) profile (g/100 g CP) in *Tenebrio molitor* (yellow mealworm), *Alphitobius diaperinus* (lesser mealworm), and *Hermetia illucens* (black soldier fly).

Item	<i>Tenebrio molitor</i> ¹			<i>Alphitobius diaperinus</i> ²			Full Fat <i>Hermetia illucens</i> ³			Defatted <i>Hermetia illucens</i> ⁴		
	N	Avg ± SD ⁵	CV ⁶	N	Avg ± SD	CV	N	Avg ± SD	CV	N	Avg ± SD	CV
Crude ash	13	4.15 ± 0.65	15.6	5	4.47 ± 0.69	15.4	15	6.56 ± 2.99	45.5	9	9.30 ± 3.27	35.2
Crude fat	14	27.0 ± 6.59	24.4	7	22.0 ± 4.87	22.1	16	34.3 ± 6.66	19.4	11	12.1 ± 6.25	51.5
Crude protein	15	56.0 ± 4.75	8.50	8	59.7 ± 10.7	18.0	17	43.1 ± 5.05	11.7	11	56.7 ± 7.16	12.6
ADF	5	8.43 ± 1.12	13.2	1	8.02		2	7.40 ± 0.12	1.69	3	7.85 ± 3.25	41.4
Chitin	4	5.02 ± 0.33	6.51	1	4.60		4	3.67 ± 1.07	29.3	6	6.11 ± 2.14	35.0
Crude fiber	4	8.20 ± 1.96	23.9	2	7.43 ± 0.14	1.92	7	7.35 ± 3.04	41.4	1	7.16	
<i>Minerals</i>												
Calcium	2	0.44 ± 0.12	28.2	3	0.50 ± 0.16	32.2	10	24.1 ± 12.8	53.2	4	38.0 ± 29.5	77.5
Phosphorus	4	7.80 ± 0.85	10.9	3	8.20 ± 0.73	8.95	10	6.01 ± 1.77	29.5	4	6.05 ± 3.19	52.7
Sodium	2	1.05 ± 0.24	23.0	2	2.21 ± 0.21	9.40	7	0.69 ± 0.21	30.0	3	0.75 ± 0.48	64.2
<i>EAA</i> ⁷												
Histidine	9	3.09 ± 0.56	18.2	7	4.32 ± 1.35	31.2	10	3.12 ± 0.52	16.8	8	2.68 ± 0.43	16.1
Isoleucine	8	4.25 ± 0.81	19.1	7	4.35 ± 0.33	7.50	10	4.37 ± 0.46	10.5	9	4.05 ± 0.58	14.4
Leucine	8	6.76 ± 1.17	17.4	7	6.64 ± 0.43	6.50	10	6.72 ± 0.57	8.40	9	6.41 ± 0.92	14.4
Lysine	9	5.60 ± 1.09	19.5	7	5.94 ± 1.04	17.5	10	6.22 ± 1.08	17.3	10	5.80 ± 1.52	26.3
Methionine	9	1.38 ± 0.37	26.7	6	1.59 ± 0.54	34.1	10	1.93 ± 0.32	16.5	10	1.61 ± 0.53	32.9
Phenylalanine	8	3.65 ± 0.64	17.6	6	4.41 ± 0.46	10.4	10	4.19 ± 0.37	8.90	7	3.41 ± 0.35	10.3
Threonine	8	3.81 ± 0.59	15.6	6	3.99 ± 0.33	8.20	10	4.19 ± 0.37	9.00	10	3.80 ± 0.62	16.3
Tryptophan	3	1.07 ± 0.38	35.5	5	1.16 ± 0.40	34.6	7	1.86 ± 0.18	9.60	5	0.82 ± 0.25	30.0
Valine	8	5.85 ± 0.98	16.8	7	5.72 ± 0.41	7.20	10	5.38 ± 0.82	15.3	9	6.46 ± 2.04	31.6
<i>NEAA</i> ⁸												
Arginine	8	5.55 ± 1.12	20.2	7	5.57 ± 0.73	13.1	9	4.93 ± 0.68	13.8	8	5.06 ± 1.88	37.1
Alanine	7	6.81 ± 0.89	13.1	6	7.02 ± 1.35	19.2	10	6.6 ± 1.14	17.3	4	6.90 ± 0.72	10.5
Aspartic acid	8	6.67 ± 2.60	39.0	6	8.30 ± 0.67	8.00	10	8.86 ± 0.83	9.30	4	7.27 ± 0.80	11.0
Cysteine	7	1.14 ± 0.81	71.3	5	1.03 ± 0.25	24.6	9	1.51 ± 1.79	119	9	0.50 ± 0.32	63.6
Glutamic acid	7	10.8 ± 1.56	14.4	6	12.4 ± 1.12	9.00	10	10.7 ± 1.57	14.7	4	10.5 ± 1.30	12.3
Glycine	8	7.29 ± 5.92	81.2	6	4.40 ± 0.22	4.90	10	5.14 ± 0.44	8.60	4	4.51 ± 0.17	3.86
Proline	7	6.31 ± 1.16	18.4	6	5.89 ± 0.52	8.90	9	5.82 ± 1.68	28.8	5	4.91 ± 0.92	18.7
Serine	7	4.14 ± 0.73	17.6	6	4.12 ± 0.39	9.40	10	4.20 ± 0.58	13.9	4	3.88 ± 0.20	5.13
Tyrosine	7	6.23 ± 1.33	21.3	5	7.76 ± 0.49	6.30	6	6.74 ± 1.96	29.1	5	4.94 ± 0.84	16.9
∑AA	7	88.0		6	91.3		10	88.4		4	79.4	
∑EAA	7	34.5		7	36.4		10	37.4		5	31.1	
∑NEAA	7	53.5		6	55.2		10	50.6		4	48.1	
EAA:NEAA	7	0.64		6	0.66		6	0.69		3	0.67	

¹ Based on literature values: [121,126–138]; ² Based on literature values: [130,139–145]; ³ Based on literature values: [39,40,121,122,128,129,146–150]; ⁴ Based on literature values: [138,148,151–156]; ⁵ Average ± standard deviation over included studies; ⁶ Coefficient of variation over included studies; ⁷ Essential amino acids; ⁸ Non-essential amino acids.

3.4. Grass Protein Meal as Compared to Soya Bean

The nutrient composition of green protein extracted from perennial ryegrass (*Lolium perenne*), lucerne (*Medicago sativa* L.), and red clover (*Trifolium pretense* L.) is shown in Table 5. Green protein produced by various biorefining methods is included, and here focus is on studies where amino acid data are available. Lucerne has previously been the forage crop of choice; however, especially in more temperate climates in Northern Europe, perennial crops like red clover and ryegrass are promising as biomass input for the biorefining process. The crude protein content of the green protein is highly dependent on process parameters: [157,158] making a direct comparison of published contents challenging. Green protein from legumes generally has a higher crude protein content than green protein extracted from grass species, levelling with soya bean meal. The crude protein in the input biomass is usually higher in legumes than in grasses, which, combined with a higher extraction efficiency in legumes [44], is expected to result in the high crude protein content of legume green protein. The amino acid composition in green protein from lucerne, red clover, and ryegrass is very similar with minor species differences within both the essential and non-essential amino acids. The conserved enzyme Rubisco

constitutes the majority of protein in plant leaves and therefore also in green protein [159]. The result is limited species variation in the amino acid composition [160]. Green protein has an EAA:NEAA ratio which for the legumes is higher than values in soya bean and fishmeal. The most abundant essential amino acid in green protein is leucine as seen with the insects and soya bean. The content of lysine, the first limiting amino acid in grain-based pig feed, is lower in green protein than corresponding values in fish meal and soya bean. Generally, the lysine content in protein from legumes is higher than values in ryegrass; however, low values in data from Baraniak [161] decrease the table average. Damborg et al. [44], Stødkilde et al. [160] presented higher values in red clover, white clover, and lucerne than in perennial ryegrass. The sulphur-containing amino acids methionine and cysteine are the limiting amino acids in protein extracted from green forage crops [160,162]. Although, green protein methionine content is exceeding soya bean methionine content, the content of cysteine in green protein is lower than in soya bean and fishmeal, making the content of sulphur-containing amino acids insufficient for poultry and pigs [68,160,163]. Green protein from ryegrass has a higher content of methionine compared to the legumes. In a grass-clover mixture, which is commonly used on arable land in Northern Europe, a large proportion of ryegrass would contribute positively to the overall methionine content.

Table 5. Chemical composition of macronutrients (g/100 g DM) and amino acids (g/100 g CP) in green protein from perennial ryegrass (*Lolium perenne*), lucerne (*Medicago sativa* L.), and red clover (*Trifolium pretense* L.)¹.

Item	Ryegrass			Lucerne			Red Clover		
	N	Avg ± SD ²	CV ³	N	Avg ± SD	CV	N	Avg ± SD	CV
Crude ash				6	10.7 ± 6.56	61.3	7	11.7 ± 4.58	39.9
Crude protein	3	28.4 ± 7.13	25.1	10	47.4 ± 10.7	15.7	3	38.7 ± 5.35	13.8
EAA ⁴									
Histidine	3	2.02 ± 0.17	8.48	7	2.21 ± 0.18	8.10	9	2.29 ± 0.29	12.5
Isoleucine	3	4.74 ± 0.36	7.50	7	4.50 ± 0.72	16.0	10	4.88 ± 0.64	13.1
Leucine	3	8.43 ± 0.53	6.32	7	7.74 ± 1.15	14.9	10	8.54 ± 0.98	11.4
Lysine	3	5.48 ± 0.56	10.2	7	4.95 ± 1.17	23.6	10	5.70 ± 0.92	16.1
Methionine	3	2.04 ± 0.19	9.31	7	1.76 ± 0.22	12.5	10	1.85 ± 0.27	14.7
Phenylalanine	3	6.17 ± 0.95	15.3	7	5.54 ± 0.65	11.8	10	5.57 ± 0.76	13.7
Threonine	3	4.65 ± 0.33	7.00	7	4.16 ± 0.78	18.8	10	4.66 ± 0.61	13.0
Tryptophan	—	—	—	—	—	—	—	—	—
Valine	3	6.27 ± 0.54	8.56	7	5.21 ± 0.97	18.6	10	6.01 ± 0.66	11.0
NEAA ⁵									
Alanine	2	6.73 ± 0.70	10.4	4	5.70 ± 0.55	9.73	9	5.80 ± 0.61	10.5
Arginine	3	5.73 ± 0.39	6.81	7	4.61 ± 1.43	31.0	10	5.54 ± 0.77	13.9
Aspartic acid	2	8.72 ± 0.70	8.03	5	9.24 ± 2.01	21.7	9	10.2 ± 1.72	16.9
Cysteine	3	0.83 ± 0.11	13.6	7	0.99 ± 0.21	21.0	9	0.67 ± 0.24	36.0
Glutamic acid	2	9.74 ± 0.79	8.13	5	8.62 ± 1.40	16.2	9	10.6 ± 1.65	15.6
Glycine	3	5.42 ± 0.31	5.64	7	4.58 ± 0.83	18.1	10	5.16 ± 0.64	12.5
Proline	2	4.63 ± 0.46	9.94	7	3.27 ± 0.83	22.3	9	4.51 ± 0.26	5.84
Serine	3	4.34 ± 0.20	4.56	7	4.72 ± 0.52	10.9	10	4.31 ± 0.41	9.61
Tyrosine	—	—	—	1	1.91	—	3	4.25 ± 0.92	21.6
Met+Cys	3	2.87 ± 0.30	22.9	7	2.75 ± 0.28	33.4	9	2.45 ± 0.56	22.9
∑AA		85.92			80.15			90.49	
∑EAA		39.80			36.06			39.50	
∑NEAA		46.13			44.09			50.99	
EAA:NEAA		0.86			0.82			0.77	

¹ Based on data from [4,160,161,164–171]; ² Average ± standard deviation over included studies; ³ Coefficient of variation over included studies; ⁴ Essential amino acids; ⁵ Non-essential amino acids.

Data on the mineral composition of green protein from grasses and legumes are very limited. However, the methods by which proteins are isolated from green juice seem to influence the mineral content of the green protein. Baraniak [161] demonstrated that the green protein from lucerne was characterized by a high content of macro- and microelements, generally exceeding the values in the lucerne plant; however, precipitating the proteins with acidification led to a low content of calcium and potassium, likely due to the minerals being distributed to the brown juice.

4. Animal Studies

In this section, studies that focus on feeding monogastric animals with meal from mussel, starfish, seaweed, insects, and forage crop protein extract will be discussed in terms of nutrient digestibility and animal performance, i.e., gain, feed intake and feed efficiency. Studies that have used conventional as well as organic monogastric production will be reviewed. Studies using conventionally held monogastrics can give an insight into the potential and limitations of alternative feed ingredients to replace high protein feed ingredients, soya bean, and fishmeal, which can be useful for organic production as well. This indicates whether they can be considered high quality protein and therefore be used to fulfil protein and amino acid requirements in organic monogastric nutrition. However, it should be taken into account that crystalline AA are used in conventional diets. Moreover, in organic animal husbandry, slow-growing breeds with generally lower nutrient requirements are used [124]. The number of studies on the reviewed feed ingredients that use slow-growing, organic monogastrics is, however, limited.

4.1. Mussel Meal and Starfish Meal

4.1.1. Digestibility

The standardized ileal digestibility of protein from both deshelled mussel meal and starfish meal has been evaluated in growing pigs [74]. A standardized ileal digestibility coefficient of 0.83 was obtained for crude protein from mussel meal, and comparable standardized ileal digestibility coefficients were found for AA. Similarly, starfish meal had a standardized ileal CP digestibility coefficient of 0.80. This indicates that protein from mussel and starfish meal will be highly available for growth. Fishmeal is characterized as having an apparent ileal N digestibility of 0.82 in growing pigs [172] and a standardized ileal CP digestibility of 0.84 in weaned pigs [173]. No studies have looked at the specific digestibility of mussel meal or starfish meal as a feed ingredient in poultry. Nevertheless, the digestibility of diets containing up to 12% mussel meal has been investigated in laying hens. Diets containing 4, 8, or 12% mussel meal compared with 3.7% fishmeal were found to give higher apparent excreta digestibility of fat but did not change digestibility of organic matter in laying hens. These results point to mussel meal in diets being as digestible as fishmeal. This would also be expected based on the comparable nutrient composition with fishmeal.

4.1.2. Growth Performance Mussel Meal

An overview of animal performance studies using mussel meal and starfish meal is presented in Table 6.

Table 6. Summary of performance characteristics of monogastric animals fed varying inclusion levels of starfish meal or mussel meal.

Type	Inclusion %	Control	Animal/Breed	Parameters	Effect	Reference
Starfish	4, 8	Fishmeal, Org. ¹	Laying hen/ Hisex white	Bird weight, FCR ² , ADFI ³ , Egg weight, Laying %	No effect No effect No effect No effect No effect	[174]
Starfish	0, 5, 10	Fishmeal	Weaned pig/ Danish Landrace/Yorkshire × Duroc	ADG ⁴ , FCR, ADFI,	Reduced 10 Worsened 10 No effect	[63]
Starfish	0, 5, 7.5, 10	Fishmeal	Weaned pig/ Danish Landrace/Yorkshire × Duroc	FCR, ADG, ADFI	Worsened 7.5, 10 Reduced 7.5, 10 No effect	[78]

Table 6. Cont.

Type	Inclusion %	Control	Animal/Breed	Parameters	Effect	Reference
Mussel	0, 4, 8, 12	Fishmeal, Org.	Laying hen/ Hisex white	ADFI, FCR, Egg weight, Laying %	No effect No effect No effect No effect	[174]
Mussel	0, 3, 6, 9	Fishmeal, Org.	Laying hen/ Lohmann Selected leghorn	FCR, Egg weight, Laying %	No effect No effect No effect	[75]
Mussel	0, 3.5, 7.0	Fishmeal, Org.	Laying hen/ Lohmann Selected leghorn and Hyline white W-98	ADFI, Bird weight, FCR, Egg weight, Laying %	No effect No effect No effect No effect No effect	[175]
Mussel	0, 3, 6, 9, 12	Fishmeal	Broiler/ Ross 308	ADFI, FCR, Bird weight	No effect No effect No effect	[176]
Mussel	0, 5	Commercial diet	Grower-finisher pig/ Yorkshire × Hampshire × Duroc	ADG	No effect	[177]

¹ Organic diet; ² Feed conversion ratio, i.e., kg feed/kg gain for growing animals and kg feed/kg egg for laying hens; ³ Average daily feed intake; ⁴ Average daily gain.

In accordance with the observation of high available protein, Wallenbeck et al. [177] found that including 5% deshelled mussel meal in diets for grower-finisher pigs gave similar weight gain compared with feeding a commercial diet. There is an overall agreement among studies using mussel meal for laying hens that there is no difference in growth performance or egg laying performance when feeding organic diets containing fishmeal or between 3% and 12% mussel meal [75,174,175]. Furthermore, there is an overall agreement that egg quality is not altered when feeding mussel meal instead of fishmeal in terms of egg weight, shell strength, or deformation, and albumen weight and height. Yolk pigmentation is found to be more orange when feeding mussel meal [174]. This effect is related to the high carotenoid levels in mussel meal. One study has been performed using conventional broiler chicks in which birds were fed no mussel meal or fishmeal, or diets with 3, 6, 9, or 12% of either fishmeal or mussel meal [176]. None of these inclusion levels were found to have affected feed intake, live weight, or feed conversion ratio (FCR) of broilers. A limiting factor for the inclusion of mussel and starfish meal could be an off-flavor and an off-odor. Indeed, 12% mussel meal was found to give a fishy taint, which was hypothesized to be due to an accumulation of methylamine [174]. However, it will still be possible to completely substitute fishmeal in commercial diets because inclusion rate of fishmeal is usually below 5%. Since blue mussels are filter feeders, the uptake of water contaminants may affect safety and toxicity of mussel meal [69,178]. Similarly, starfish, who mainly feed on mussels, could be affected. In the EU, however, the quality of shellfish water is regulated continuously because of EU Directive 2006/113/EC [179]. Moreover, Jönsson and Holm [180] fed either 15% normal mussel meal or 15% mussel meal with high levels of the diarrhetic shellfish toxin, okadaic acid, or a commercial diet to conventionally held laying hens and found no difference in growth and egg laying performance of hens. The level of okadaic acid in egg yolk was moreover not different between diets.

4.1.3. Growth Performance Starfish Meal

In a two-week study, Sørensen and Nørgaard [63] showed that including 5% starfish meal in weaned pigs' diets gave similar daily gain and feed efficiency compared with similar levels of fishmeal or extruded soya bean. On the other hand, increasing the inclusion level to 10% reduced both gain and feed efficiency. This can be related to a high calcium concentration in starfish meal. Excess calcium can limit the availability of phosphorus by binding phytate, competing for the active sites of phytase or by increasing intestinal pH and thereby reducing enzyme efficacy [181,182]. Indeed, phosphorus levels

in plasma of pigs fed 10% starfish meal compared with pigs fed fishmeal or 5% starfish meal were reduced [63]. Phosphorus is important for growth as it plays a role in energy metabolism, cell differentiation, and bone development [183,184]. Additionally, the unabsorbed phosphorus will be excreted into the environment. In a subsequent study at a commercial setting, the same conclusions were obtained when including 5, 7.5, or 10% starfish meal in diets of newly weaned conventionally held pigs in which 7.5% starfish meal also lowered gain and feed efficiency [78]. Higher levels of starfish meal can likely be included in laying hen ratios. It was indeed found that bodyweight, FCR, and feed intake of laying hens were unaltered by the inclusion of either 4 or 8% starfish meal as a fishmeal substitute [175]. Moreover, egg laying rate and egg weight did not differ when feeding starfish meal or fishmeal [175]. Although, layers can consume high levels of calcium to sustain egg production, the inclusion ratio of starfish meal in complete feed will still be limited by the calcium content. The requirement for high levels of calcium is, namely, time dependent, and calcium included above the required level, and outside the period of egg shell formation, may not be effectively utilized by laying hens, therefore risking higher excretion of phosphorus as a result of bone mineralization [185].

4.2. Insect Meal

4.2.1. Digestibility

The effect of insect-containing diets on digestibility is summarized in Table 7.

Table 7. Nutrient digestibility of monogastrics' diets containing varying inclusion levels of insect meals (*Hermetia illucens*, HI and *Tenebrio molitor*, TM) as compared to a basal diet or diets containing soya bean.

Nutrient	Insect	Inclusion %	Control	Animal	Description	Effect Insect	Reference
CP, fat, DM/OM	HI defat.	0, 5, 10	Soya bean	Weaned pig	ATTD ¹	No effect	[186]
CP, fat, DM/OM	HI full/defat.	4–8	Soya bean	Weaned pig	ATTD/AID ²	No effect	[187]
DM/OM	HI defat.	0, 7.3, 14.6	Soya bean	Laying hen	AID	Reduced at 14.6	[188]
CP, DM/OM	HI defat.	17	Soya bean	Laying hen	AID	Reduced	[151]
CP	HI defat. (Met:Cys balanced)	20	Soya bean	Broiler	AID	No effect	[152]
CP	HI full/defat.	25	Basal	Broiler	ATTD	Reduced	[189]
AA	TM	0, 5, 10	Soya bean	Growing pig	Ileal dig.	Reduced at 10	[190]
CP, DM/OM	TM	30	Soya bean	Broilers	AID	Reduced	[126]

¹ Apparent total tract digestibility; ² Apparent ileal digestibility.

Several studies suggest that the effect of insects on digestibility is dose-dependent. Both Biasato et al. [186] and Spranghers et al. [187] found that apparent total tract digestibility of dry matter, organic matter, crude protein, and crude fat was not different when feeding between 4% and 10% black soldier fly larvae or soya bean to newly weaned pigs. The same conclusions were drawn for digestibility of nutrients in the ileum [187]. Furthermore, numerically lower AA digestibilities were found when feeding growing pigs 10%, but not 5%, yellow mealworm compared to soya bean [190]. In line with this, at 14.6% compared to 7.3 or 0% inclusion of defatted black soldier fly, the apparent ileal DM and organic matter (OM) digestibilities were lowered in laying hens [188]. The CP digestibility reduced linearly with increasing level of insect meal. High inclusion levels almost always give a reduced nutrient digestibility. In line with this, both full fat and defatted black soldier fly gave lower uric acid-corrected apparent total tract CP digestibility when replacing 25% of a basal diet containing soya bean for Ross 308 broilers [189]. Furthermore, when feeding Shaver brown broilers 30% yellow mealworm, Bovera et al. [126] found that ileal digestibility of DM, OM, and CP was higher for birds fed diets based

on soya bean than yellow mealworm meal. Cutrignelli et al. [151] also observed that laying hens fed 17% defatted black soldier fly had lower apparent ileal digestibility of DM, OM, and CP than hens fed diets containing soya bean. The nutrient composition of insects can affect protein digestibility: in an in vitro study, it was found that crude protein digestibility of black soldier fly and yellow mealworm was negatively correlated with their chitin content [121]. Removal or reduction of chitin may improve protein quality of insects. Some studies indicate that the level of sulfuric amino acids may be of importance. The inclusion of at least 20% defatted black soldier fly in broiler diets with a balanced ratio of methionine and cysteine did not give different ileal digestibilities of CP or EAA compared to a diet containing soya bean [152]. Additionally, Jensen et al. [130] found sulphuric AA to be most limiting in a rat study. Furthermore, the effect of insect meal on digestibility seems dependent on the type of insect. De Marco et al. [129] observed lower apparent ileal digestibility of the mean of AA when substituting 25% of a basal diet for broilers with full fat black soldier fly compared to yellow mealworm.

4.2.2. Growth Performance

Table 8 contains an overview of the effects of including insect meals on growth performance of monogastric animals. Most studies do not find any differences in growth performance parameters of pigs when supplementing up to 10% insect meal (Table 9). There was no effect on body weight gain or feed efficiency of weaned pigs when substituting soya bean with either 5 or 10% defatted black soldier fly [186]. Neither was there an effect on growth performance when feeding 4 or 8% full fat or 5.4% defatted black soldier fly meal to weaned pigs instead of a control diet [187]. In finishing pigs, no difference in gain or feed efficiency was observed when feeding 8% black soldier fly compared with a control diet based on soya bean and maize [191]. In the same study, feeding 4% black soldier fly increased gain and feed efficiency. A concentration of 5 or 10% yellow mealworm did give equal feed efficiency and feed intake in male grower pigs compared with feeding soya bean [190]. Daily gain, however, was reduced when feeding 10% yellow mealworm. This coincides with a lower AA digestibility for this diet. In another study, using weaned pigs, including 2%, but not 1 or 4% black soldier fly, was found to improve feed efficiency and gain compared with fishmeal [192]. None of the above-mentioned studies found a difference in feed intake.

Table 8. Summary of performance characteristics of monogastric animals fed varying inclusion levels of insect meal (*Hermetia illucens*, HI and *Tenebrio molitor*, TM).

Insect	Inclusion %	Control	Animal	Description	Effect Insect	Reference
TM	0, 5, 10	Soya bean	Growing pig/Piétrain × German Landrace × German Edelschwein	FCR ¹ , ADFI ²	No effect No effect	[190]
TM	0, 5, 10	Soya bean	Growing pig	ADG ³	Reduced at 10	
HI defat.	0, 5, 10	Soya bean	Weaned pig Topigs	ADG, FCR, ADFI	No effect No effect No effect	[186]
HI full/defat.	4–8	Soya bean	Weaned pig	ADG, ADFI, FCR	No effect No effect No effect	[187]
HI full fat	0, 4, 8	Soya bean	Finishing pig/Duroc × Landrace × Large White	ADG, FCR	No effect at 8 Improved at 4	[191]
HI full fat	0, 4, 8	Soya bean	Finishing pig	ADFI	No effect	[191]
HI full fat	0, 1, 2, 4	Fishmeal	Weaned pig/Duroc × Landrace × Large White	FCR, ADG	Improved at 2 Increased at 2	[192]
HI full fat	0, 1, 2, 4	Fishmeal	Weaned pig	ADFI	No effect	[192]

Table 8. Cont.

Insect	Inclusion %	Control	Animal	Description	Effect Insect	Reference
TM	0, 5, 10, 15	Soya bean	Broiler/Ross 708	ADFI	L ⁴ increase d. 1–25	[127]
TM	0, 5, 10, 15	Soya bean	Broiler	ADG	L increase d. 1–12 No effect d. 12–25	[127]
TM	0, 5, 10, 15	Soya bean	Broiler	FCR	L increase d. 12–25 No effect d. 1–12	[127]
TM	0, 5, 10, 15	Soya bean	Broiler	FCR, ADFI, ADG	No effect d. 25–40 No effect d. 25–40 No effect d. 25–40	[127]
TM	7.5	Gluten meal	Broiler/Hubbard hybrid JA57/S77CN (slow growing)	ADFI, FCR, BW	No effect No effect No effect	[193]
TM	8.0	Soya bean	Broiler/Ross 308	ADFI, FCR	Reduced Improved	[194]
TM	8.0	Soya bean	Broiler	ADG	Increased	[194]
TM	30	Soya bean	Broiler/Shaver brown	FCR	Reduced	[195]
TM	30	Soya bean	Broiler	FCR, ADFI	No effect d. 30–45 Reduced d. 45–62	[195]
TM	30	Soya bean	Broiler	ADG	No effect d. 30–45 No effect d. 45–62	[195]
HI defat	0, 5, 10, 15	Soya bean	Broiler/Ross 708	Liveweight, ADG	L and Q ⁵ effect L and Q effect	[196]
				ADFI	L and Q d. 1–10	
				FCR	No effect d. 10–35 No effect d. 1–10 L effect d. 10–35 Q effect d. 10–24	
HI defat	ca. 20	Soya bean	Broiler/Ross 308	FCR	No effect d. 1–21 Reduced d. 21–35	[152]
HI defat	ca. 20	Soya bean	Broiler	ADG	No effect d. 1–21 Reduced d. 21–35	[152]
HI defat	ca. 20	Soya bean	Broiler	ADFI	Reduced d. 1–21 No effect d. 21–35	[152]
HI defat.	0, 7.8	Soya bean Org. ⁶	Laying hen/Hubbard S757 (slow growing)	ADG	No effect	[197]
HI defat.	0, 7.3, 14.6	Soya bean	Laying hen/Hy-line Brown	Egg weight, Bird gain, FCR, Laying %	No effect No effect No effect Increased 7.3	[188]
HI defat.	0, 12, 24	Soya bean Org.	Laying hen/Lohmann Selected Leghorn	Laying %, ADFI	No effect No effect	[146]
HI defat.	17	Soya bean Org.	Laying hen/Lohmann Brown Classic	Live weight	Reduced	[151]
HI defat	17	Soya bean Org.	Laying hen/Lohmann Brown Classic	ADG, ADFI, FCR	Reduced Reduced Reduced	[198]
				Egg weight, Laying %	Reduced Reduced	

¹ Feed conversion ratio, i.e., kg feed/kg gain for growing animals and kg feed/kg egg for laying hens; ² Average daily feed intake; ³ Average daily gain; ⁴ Linear; ⁵ Quadratic effect; ⁶ Organic diet.

Similar to pigs, the effect of feeding insect meal on growth performance seems dose-dependent in poultry. In a dose-response study, in which 0, 5, 10, or 15% defatted black soldier fly was fed to broilers [196], maximum live weight and gain were observed at 10% inclusion, and a drop for both parameters was observed when exceeding this level.

Moreover, during the last 10 growing days, gain decreased linearly with increasing levels of insect meal. FCR was affected both linearly and quadratically over the total growing period with the highest value found at 15%. Conversely, when broilers were fed a diet containing around 20% defatted black soldier fly or soya bean with equal levels of methionine and cysteine, their gain and FCR were not different during the first three weeks after hatching [152]. It should be noted, however, that several EAA were supplied at higher levels in the soya bean diet of this study. Feed intake, in this study, was reduced when feeding insect meal compared to feeding a diet with soya bean. After the initial three weeks, neither gain nor feed intake was different, but FCR was reduced when feeding insect meal.

In a dose-response study, feeding 0, 5, 10, or 15% yellow mealworm as a partial substitute of corn and soya bean has been found to linearly increase feed intake of broilers over the majority of their growing period [127]. Gain, however, was only linearly improved during the starter period, but not during the subsequent two weeks. This is related to the observed increase in FCR with increasing levels of yellow mealworm. In another study, feeding 7.5% yellow mealworm at the expense of gluten meal to free-range broilers did not affect any growth performance parameters [193]. In contrast, FCR and feed intake were found to be lower when feeding Shaver brown broilers 30% yellow mealworm compared with feeding soya bean in the last two weeks of the growing period [195]. Furthermore, ingested protein from yellow mealworm was found to be more efficiently utilized for growth. The authors related these results to a feed intake reduction when feeding yellow mealworm during this period. In a subsequent study, using the same diets, a reduction in ileal digestibility of crude protein was found at the end of the growing period when feeding insect meal [126]. Feed intake and body weight gain were not different during the overall growing period. On the other hand, when broilers were fed 8% yellow mealworm, an overall reduction in feed intake and an increase in gain as well as a reduction in FCR were obtained [194]. These results may have been confounded by a 39% higher calculated methionine concentration in the insect supplemented diet. Methionine is an EAA that affects the potential for body protein deposition and subsequently determines growth.

Laying hens that ate a diet containing 17% black soldier fly had lower live weights, which could be related to lower digestibility of the insect diet [151]. In another study, it was found that neither soya bean nor 7.3 or 14.7% black soldier fly meal affected egg weight, bird gain, feed intake, or FCR (per gram of egg) of laying hens [188]. Laying percentage, however, was higher when birds were fed 7.3% black soldier fly as a partial substitute for SBM. Lohmann Brown laying hens gained and ate less when eating 17% black soldier fly than soya bean [198]. Egg weight, egg mass, and laying percentage were also lower. The FCR, however, expressed as gram of feed ingested per gram of egg, was lower. In relation to a much greater reduction in gain than the reduction in feed intake, nutrients may have been prioritized for egg formation. Feeding laying hens with either 12 or 24% black soldier fly meal instead of soya bean did not affect laying percentage, feed intake, or weights of the different components of the eggs laid [146]. Although, this may have been because the crude protein level in the high black soldier fly diet in this study was 1.4% higher than the control. Finally, when Hubbard s757 hens were fed organic diets with or without 7.8% defatted black soldier fly, growth performance did not differ [197].

4.3. Green Protein Meal

4.3.1. Digestibility

The digestibility of green protein is summarized in Table 9. Most studies are performed on rats where digestibility of protein and the biological value have been evaluated. High fecal digestibility of green protein extracted from lucerne, red clover, white clover, and perennial ryegrass was recently reported in rats [160,163] with standardized N digestibility of up to 88%, which is similar to published values for other plant-based food products [199]. The extraction of protein from the plant fiber matrix significantly improved N digestibility compared to the residual pulp [160,163] and for lucerne also compared to the unfractionated plant [160]. The digestibility is highly dependent on input plant material and on the process

parameters. Szymczyk et al. [200] reported higher protein digestibility and biological value in Italian ryegrass (*Lolium multiflorum*) (79% and 58%, respectively) compared to red clover (62% and 31%, respectively). Higher digestibility and biological value in grasses (85% and 58%, respectively) compared to legumes (65% and 45% for lucerne, 80% and 44% for red clover) was found by Maciejewicz-Rys and Hanczakowski [168] confirming differences in digestibility between species. Moreover, within a plant species, different varieties and plant development stages will influence green protein quality. This is seen in lucerne, where green protein extracted from low saponin-containing varieties had higher digestibility than green protein from high saponin lucerne [201]. During plant maturity, the content of fiber increases while the content of protein decreases [202,203]. This will affect protein extractability [159] but also gross protein value [204]. The method used for precipitation of green protein from the juice will influence digestibility [200,205–207], highlighting the importance of carefully selecting input biomass and processing method in order to maximize quality.

The ileal digestibility of amino acids was reported using green protein extracted from red clover and perennial ryegrass [208]. Standardized ileal digestibility for all indispensable amino acids ranged from 62 to 81%, except for cysteine which ranged as low as 13–37%, all significantly lower than of reference soya bean meal. The green protein used had crude protein contents of 33–38% of DM [208], suggesting a high content of fiber which is known to co-precipitate with the protein [45]. A higher crude protein content of the green protein through improved harvest and processing technology may increase the nutritional quality. The improvement in digestibility is required for green biomass to be both environmentally and economically competitive as feed protein [208].

Table 9. Nutrient digestibility of diets containing protein extracted from forage crops as compared to diets containing a control ingredient when fed to monogastric animals.

Biomass	Inclusion %	Control	Animal	Description	Effect	Reference
Red clover <i>Trifolium pratense</i> L.	100	Soya bean	Pig	AID ¹ SID ²	Reduced	[208]
Perennial Ryegrass <i>Lolium perenne</i>	100	Soya bean	Pig	AID SID	Reduced	[208]
Red clover	100	–	Rat	TD ³ ; BV ⁴	64; 51	[206]
Red clover	100	–	Rat	TD; BV	62; 31	[200]
Italian ryegrass <i>Lolium multiflorum</i>	100	–	Rat	TD; BV	79; 58	[200]
Ryegrass	100	Lactalbumin	Rat	RNV ⁵ ; PTD ⁶	Reduced	[209]
Lucerne <i>Medicago sativa</i> L.	100	Lactalbumin	Rat	RNV; PTD	Reduced	[209]
White clover <i>Trifolium repens</i>	100	Lactalbumin	Rat	RNV; PTD	Reduced	[209]
Lucerne	100	–	Rat	TD; BV	86; 77	[210]
Red clover	–	–	Rat	TD; BV	70.6; 53.7	[211]
Lucerne	100	–	Rat	TD; BV	76–88; 37–57	[207]
Lucerne	–	–	Rat	TD; BV	65; 45	[168]
Red clover	–	–	Rat	TD; BV	80; 44	[168]
Italian ryegrass	–	–	Rat	TD; BV	85; 58	[168]
White clover	100	–	Rat	TD	83.0	[162]
Lucerne	–	–	Rat	TD	85.0	[160]
Red clover	–	–	Rat	TD	77.4	[160]
Perennial Ryegrass	–	–	Rat	TD	75.4	–
White clover	–	–	Rat	TD	79.3	[160]

¹ Apparent ileal digestibility; ² Standardized ileal digestibility; ³ Total tract digestibility; ⁴ Biological value; ⁵ Relative nutrient value; ⁶ True protein digestibility.

4.3.2. Growth Performance

Nutritional studies on green protein have mainly focused on lucerne as the raw material where the nutritive value and effect on growth of including lucerne plant juice or green protein to diets of poultry, pigs, or rats have been investigated (Table 10). The results have been inconsistent [212], likely due to improper processing and variations in the methods used [158,212].

In a feeding trial with broilers, Szymczyk et al. [200] substituted soya bean meal with green protein from red clover and reported increased average daily feed intake (ADFI) in broiler chicks in the starter period but a decreased ADFI in the finisher period. Despite an increased ADFI, the average daily gain (ADG) in the starter period was reduced, indicating a quality of green protein inferior to soya bean meal. Similarly, reduced weight gain and FCR were seen in another study where 30% and 40% of red clover green protein was included in broiler diets [213]. On the contrary, including green protein from Italian ryegrass in the diets for broilers had no effect on performance [200]. Including green protein from lucerne in broiler diets improved performance when the lucerne was a low saponin variety; however, reduced performance was seen with green protein from high saponin varieties [213]. Green protein from a mixture of grass and clover was included in the diets of the organic broilers and caused a reduced overall ADFI and slaughter weight for broilers on a diet containing 24% grass-clover protein extract. The ADG and FCR of the broilers receiving 8% grass-clover protein extract did not differ from the control during the feeding trial. The reduced performance was explained by insufficient content of EAA and a high content of protein being bound to insoluble complexes [45].

In pigs, several studies have reported inclusion of lucerne green protein without adverse effects on pig performance [205,214–217]. Hsu and Allee [217] and Sugimoto et al. [217] substituted up to 100% of the soya bean meal with green protein from lucerne, and generally the inclusion had no effect on ADG, ADFI, and FCR. However, Hsu and Allee [216] demonstrated increased weight gain in finisher pigs fed diets where 66 and 100% of the soya bean meal was substituted. Similar results were observed by Pietrzak and Grela [215] where sows in late lactation had a higher weight when lucerne (1.5–3.0%) was included in the diets. Feeding trials with other plant species are limited and have primarily been performed with inclusions of green juice and not green protein [218–221]. The results are inconclusive, and challenges with excessive mineral levels in the juice [164] and palatability have been reported [221]. As with digestibility, process parameters will affect performance exemplified by the higher performance in pigs fed freeze-dried lucerne green protein than in pigs fed commercial lucerne protein concentrate (X-Pro) produced by drying at higher temperatures [205]. During production of the green protein, lipids in the plants are concentrated together with the extracted protein [222]. The fatty acid (FA) composition in green protein is dominated by the unsaturated omega-3 fatty acid alpha-linolenic acid, which is of nutritional importance; however, due to susceptibility to oxidative stress, the presence of unsaturated fatty acids in green protein renders the extracted protein and the final meat product more vulnerable to rancidity. Moreover, the reactive radicals produced during oxidation may pose health issues for the animals during feeding [159]. Feeding broilers with green protein produced from grass-clover resulted in an increased deposition of alpha-linolenic acid in both breast meat and fat tissue [45] and also increased the level of yellow-colorization. In an experiment with pigs, Carr and Pearson [223] reported that lucerne green protein affected some carcass parameters such as depth of eye muscles, fat, and skin. A reduced incidence of diarrhea and diarrhea-related mortality was reported when piglets were fed 1.5 and 3.0% green protein from lucerne [215]. Kwiatkowska et al. [224] demonstrated that including 1.5 and 3% green protein from lucerne in the diet for broilers not only increased body weight at day 21 but also reduced mortality rate. Moreover, the bone mechanical strength was improved, possibly related to bioactive compounds in lucerne. The animals had increased slaughter yield, increased pectoral muscle weight, and reduced abdominal fat.

Table 10. Summary of performance characteristics of monogastric animals fed varying inclusion levels of protein-extracted forage crops.

Biomass	Inclusion %	Control	Animal	Description	Effect	Reference
Red clover <i>Trifolium pretense L.</i>	1:1 SBM protein ratio	Soya bean	Broiler/Astra B	ADFI ¹ , ADG ²	Increased w. 0–4, Reduced w. 5–8 Reduced w. 0–4 No effect w. 5–8	[200]
Italian ryegrass <i>Lolium multiflorum</i>	1:1 SBM protein ratio	Soya bean	Broiler	ADFI, ADG	No effect	[200]
Lucerne/Italian rye-grass/White clover <i>Trifolium repens</i>		Casein	Broiler Rode Island Red × Light Sussex	Weight, Gross protein value	Reduced. Dependent on plant maturity	[225]
Red clover	30, 40		Broiler H&N “Meat Nick”	Weight gain, FCR ³	Reduced	[213]
Grass-clover mix ⁴	0, 8, 16, 24	Soya press cake Org. ⁵	Broiler Color Yield JA57 (slow growing)	ADFI, ADG, FCR	No effect at 8 Reduced at 16–24	[45]
Lucerne <i>Medicago sativa L.</i>	1.5, 3.0	Soya bean	Broiler Ross 308	ADFI, ADG, FCR	Reduced Increased BW d. 21 No effect	[224]
Lucerne (low+high saponin; LS/HS)	10, 20, 30, 40	Soya bean	Broiler Layer Leghorn	Weight gain, FCR	LS improved HS: reduced Reduced when 30 and 40% wet undried LS alfalfa protein concentrate was incorporated in the diets.	[213]
Lucerne	0, 5, 10, 15, 20	Soya bean	Pig	ADG, FCR	Good performance in all groups	[214]
Lucerne	–	Soya bean	Pig Yorkshire	ADG, FCR	No effect	[205]
Lucerne	0, 33, 66, 100 ⁵	Soya bean	Crossbred Pig	ADG, ADFI, FCR	Starter: no effect Grower: no effect Finisher: increased gain at 66 and 100%	[216]
Lucerne	0, 25, 50, 100 ⁵	Soya bean	Pig Landrace	ADG, Weight gain, FCR	No effect	[217]
Lucerne	100	Fish meal+meat and bone meal	Pig Landrace × Large White	ADG, ADFI	No effect 25–80 kg Increased	[223]
Lucerne	1.5, 3	Soya bean	Pig (sow) Polish Landrace × Polish Large White	Weight growth	Late gestation sow: weight increased, Piglet: no effect	[215]
Lucerne	10, 20, 29, 56	Soya bean	Rat	ADG	No effect at 10–20 Reduced at 29–56	[226]
Lucerne, Red clover, Italian ryegrass	100 ⁶	Effect of precipitation pH	Rat	Intake, Growth, AID ⁷	No effect	[227]
Lucerne	100	Casein	Rat	Weight, PER ⁸	Reduced	[228]

¹ Average daily feed intake; ² Average daily gain; ³ Feed conversion ratio, i.e., kg feed/kg gain for growing animals and kg feed/kg egg for laying hens; ⁴ Perennial ryegrass (*Lolium perenne L.*), hybrid ryegrass (*Lolium x boucheanum*), white clover (*Trifolium repens L.*) and red clover (*Trifolium pretense L.*); ⁵ Organic diet; ⁶ Sole protein source; ⁷ Apparent ileal digestibility; ⁸ Protein efficiency ratio.

5. Conclusions and Perspectives

The feed ingredients reviewed in this paper, i.e., starfish, mussel, insect, and green protein extracted from perennial forages, demonstrate that there are several sustainable opportunities and challenges of increasing locally available protein for organic monogastric production in Europe. Firstly, the quality and availability of protein can be elevated via extraction of protein from forage crop proteins, which moreover stimulates transition from annual to more sustainable perennial crops. Secondly, underutilized protein, i.e., waste mussel or starfish removed to prevent mussel losses, can be used. Lastly, the development of novel and sustainable industries can provide protein, in this case insect rearing on low quality unutilized biomass. The use of mussels from eutrophication mitigation sites is, moreover, of interest as it facilitates circular use of nutrients and improves water quality. This generates feed ingredients with differing protein levels of 28–66% and similar or higher EAA:NEAA ratios to conventional ingredients. All ingredients have potential to be used for monogastrics; however, inclusion of starfish and insect is limited by calcium and chitin concentration, respectively. In particular for feeding starfish and green protein, more studies on monogastrics in organic production are needed. Future research is also needed to improve quality, environmental and economic sustainability of the processing of mussel meal and green protein. A sufficient yield can be a bottleneck for the use of starfish and green protein. Legislation may be a bottleneck for the use of insect meal in organic diets for monogastrics. If insects are to be used as a sustainable feed ingredient, more knowledge on efficiency, emissions, and energy input in relation to the type of diet that insects are reared on is needed.

Finally, it should be taken into account that single feed ingredients provide only part of the required nutrients of a complete diet, and it is necessary to have a large array of different feed ingredients to compose optimal diets for monogastric animals.

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