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## Protein hunger of the feed sector: the alternatives offered by the plant world

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### ABSTRACT

The expected future demand for highly nutrient animal food products will push the animal production system to search for new sources of high-quality protein feedstuffs. In this scenario, economic and environmental issues will have to be considered while reducing the competition with the plant-based human food chains. Legume grains and some oilseed cakes, by-products from the oil industry, are the main protein sources for ruminants and terrestrial monogastrics such as pigs and poultry. Their relevant role will hold in the next decades, but it is necessary to increase the diversification of sources that can be grown profitably throughout the world, including European countries. Microalgae are a promising source of protein and other nutrients for animal feeding. However, an amazing richness of biologically active substances makes these organisms very interesting as feed ingredients, as their role go far beyond the supply of nutrients. Due to the limited usage of microalgae as human foodstuffs or food ingredients, low competition between microalgae-based feed and food chains is predictable. This review aims to synthesise current knowledge on minor pulses and other protein-rich plant products and microalgae, as alternative ingredients to the conventional animal protein sources, focussing on their production, availability, and nutritional values. Points of strength, weakness, opportunity and threat related to the use of these protein sources in animal feeding are separately analysed through a SWOT approach to underlie future needs in terms of research and/or technological development that could help valorise these nutrient sources as feed ingredients.

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## Introduction

The projected increase from 2005 to 2050 in the global demand for animal products (meat and milk) has been estimated to be between 48 and 57% (Alexandratos and Bruinsma 2012), whereas animal protein demand will grow even more. The meat production from poultry, swine, beef, and dairy products should double, whereas fish production should almost triple by 2050. Such an increase in livestock

production will generate an increase in feed supply, estimated to be more than 1.3 billion tons of dry matter. In 2018, the global animal feed production was estimated at 1.103 billion tons, corresponding to a value of more than US\$400 billion. The EU 28 contributed for 277 million tons, a lower quantity in comparison to the values registered for Asia-Pacific region (394.9 million tons) but higher than values registered for North America (198.9 million tons); China, the

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United States, Brazil, Russia, India, Mexico, Spain and Turkey together produced 55% of the world's feed production (Alltech 2019).

Proteins are the most expensive and more limiting ingredients in feed formulation. In the EU, roughage (grass and silage maize) is the main source of feed protein, representing 45% of the total feed, while oil-seed meals and cereals cover the 26 and 24%, respectively. By considering the classification of feed based on the protein content in LowPRO feed (less than 15% of protein content), MiddlePRO feed (15–30% of protein content), HiPRO feed (30–50% of protein content) and SuperPRO feed (with over 50% protein content), the EU Feed Protein Balance sheet shows self-sufficiency rate for 97, 75, 29 and 92%, respectively. Overall, about 80% of the total EU use of feed sources has EU origin and dependency lays on imports for the HiPRO category of feed sources (<https://iffif.org/global-feed/statistics/>). As a precaution after the Bovine Spongiform Encephalopathy outbreak, the EU banned the use of processed animal proteins (PAPs) such as meat meal, meat and bone meal, poultry meal, etc. in animal feeds (Reg (EC) 999/2001; European Commission 2001). This led to an increase of the use and cost of the plant protein sources. The ban was partially lifted (Reg (UE) 56/2013; European Commission 2013) when PAPs were reintroduced for aquafeeds. After about 20 years, the European Union is considering lifting this ban also for poultry and pigs. This would undoubtedly ease the cost of protein-rich feedstuffs. At the global level, the main source of protein for feed until now has been the soybean meal, derived by the oil extraction process. Due to the unsustainability of the utilisation of soybean products for feeding animals instead of feeding humans, the approach adopted so far threatens the food supply of future generations and the objective of the recent and future research will be to find new feeds, new ingredients for feed and new protein sources for animal production, not in competition with humans.

Poultry, swine and ruminant sectors absorb the 42, 27 and 19% of the total compound of the feed production, respectively (Alltech 2019), even though ruminants, poultry and pigs supply the 45, 31 and 20% of animal proteins for humans, respectively (Mottet et al. 2017).

Currently, aquafeeds represent a limited percentage (4%) of the global feed production, but this sector will expand considerably by 2050 being the second only to the poultry one (Hua et al. 2019). The number of commercial aquafeeds amounted to 49.7 million tons in 2015 and it is estimated to increase to 87.1 million tons in 2025 (Hua et al. 2019). However, this

forecasting is considered an underestimate not including the farm-made feeds (Tacon and Metian 2015). Another aspect worthy of consideration is the higher digestible protein content that characterises aquafeeds compared to the feed for terrestrial livestock, ranging from 30 to more than 50% according to species and life stage (Craig 2017; Yarnold et al. 2019). The potential of plant-derived proteins must, in the case of aquaculture, also take into account possible scarce palatability and the presence of anti-nutritional factors, and the very variable, and sometimes sub-optimal, protein levels. The option offered by the plant-derived proteins represents an opportunity to maintain the evolution trend of aquaculture despite the stagnant supply and the increase of the price of fishmeal, that is the major and the optimal high protein feedstuff for aquafeeds (Kim et al. 2019). To balance food, feed and biofuel industries requests, novel protein sources for feeding farmed animals, characterised by high sustainability are mandatory.

In this review current knowledge on minor pulses and other protein-rich plant products and microalgae, as alternative ingredients to the conventional animal protein sources, are synthesised focussing on their production, availability, and nutritional values. In addition, through a SWOT analysis, points of strength, weakness, opportunity and threat of the use of these protein sources in animal feeding are separately analysed.

## Protein-rich plants

### Global availability of minor protein-rich plants

Official data on the availability of protein-rich plants and/or seeds are regularly updated by the Statistical Division of the Food and Agriculture Organisation and can be searched by using a web-based interface (FAOSTAT). Unfortunately, not all the data regarding production, yield and harvested area for plant species, especially those high in proteins, are searchable such as, for example, several protein sources belonging to the 'pulses nes' category (*Dolichos* spp., *Canavalia* spp., *Psophocarpus tetragonolobus*, *Cyamopsis tetragonoloba*, *Stizolobium* spp., *Pachyrrhizus erosus*) that are reported jointly with no specifications about the addressed market (i.e. food vs. feed). Among the protein-rich plants, legumes play a relevant role in farm animal feeding both as seeds (grain legumes or pulses) and as plants (fresh and conserved forages). In this review, the only source of protein that can be regarded as 'concentrates' will be considered further. Overall, the global pulses production rose from 2007

to 2017 with an average yearly increase of about 3.4 million tons. Also, the grain legumes production has increased in Europe and in Italy as well and can be seen as a reactive approach with respect to the chronic deficit of plant-protein production at the EU level (Martin 2014; Lienhardt et al. 2019). Beans (*Faseolus vulgaris*), peas (*Pisum sativum*), and chick peas (*Cicer arietinum*) represent approximately two-third of the total production of pulses at the world level, while peas (dry) alone represents about 60% of pulses production at the European scale (Table 1) (Food and Agriculture Organization Statistics Division 2019). Only vetch (*Vicia* spp.) farming has registered a negative trend production at both world and European level as well as the beans and lupins (*Lupinus* spp.) production in Italy. The increased world interest in producing cowpeas (*Vigna sinensis*, *Dolichos sinensis*) and pigeon pea (*Cajanus cajan*) does not seem to have gained similar favour by the European farmers and, in the case of pigeon peas, by the Italian ones. This behaviour can be partially understood as far as the pigeon peas have a rather low yield (0.77 t/ha, averaged world yield) (Food and Agriculture Organization Statistics Division 2019) which, probably, make unsustainable its cultivation in Europe. As regards the pseudo-cereals, unfortunately, there are no official data on the world production of amaranth grain (*Amarantus caudatus*) separately from other cereal-like plants of the class 'cereals nes' (Food and Agriculture Organization 1994). The average area cultivated, the average seed production (Food and Agriculture Organization Statistics Division 2019), the estimated availability of crude proteins based on yield factors available in the literature (Bahkali et al. 1998;

Sammour 1999; Heuzé et al. 2015) and trends from 2007 to 2017 of pseudo-cereals and oil-bearing seeds outline that the production increase is partially due to the increase of acreages, such as for quinoa, and partially depends on increased yields, such as for linseed (Table 2). Among the oilseeds, above soybean, sunflower and colza, linseed and sesame represent the main sources of recoverable crude proteins, both at global and at European scale (Table 2). It is worth to note that the acreage of safflower crop at the European scale passed from 0.4 in 2007 to 154 thousand hectares in 2017, that lead to estimates of about 20,000 tons of recoverable proteins (Table 2).

### Composition and nutritive value of pulses and other plant protein sources and by-products

Overall, in the formulated feeds the amino acid profile of grain legumes, which by definition are the seeds of legumes in a dry state, is complementary to that of cereals even though, usually small amounts of essential amino acids, as methionine and lysine, must be added or supplemented to obtain a balanced diet. Chickpea (*C. arietinum*) is a good source of protein (up to 29% of dry matter, DM; lysine about 7% of crude proteins, CP) and energy for both ruminants and monogastric (Mustafa et al. 2000; Pulse Australia 2017), supporting milk, meat and/or egg production (Bampidis and Christodoulou 2011). Even though the chickpea proteins are rich in lysine, sulphur-containing amino acids and threonine are typically low. Two chickpea grain cultivars (22.5–23.5% CP on DM) were used to formulate alternative diets for pigs by substituting the 75% of the soybean, SBM (43.6% CP on DM) included in the control diet (Visitpanich et al. 1985). No significant differences between treatments and

**Table 1.** Production of main pulses' seed (1000 t) and relative trends at World, European and Italian geographical level in the last decades (FAOSTAT, 2019).

FAO Code <sup>a</sup>	FAO item	2007			2012			2017			Diff. 2007-2017 (%)		
		World	Europe	Italy	World	Europe	Italy	World	Europe	Italy	World	Europe	Italy
176	Beans (dry)	21,737.90	337.1	12.1	24,449.50	467.3	11.8	31,405.90	1,104.30	11.2	44.5	227.6	-7.4
187	Peas (dry)	9,106.00	2,655.90	40.6	10,563.80	3,328.70	24	16,205.40	7,084.80	48.5	78.0	166.8	19.5
191	Chick peas	9,690.30	75.8	6.3	11,519.40	117.9	11.2	14,776.80	560.4	33.5	52.5	639.3	431.7
181	Broad beans (dry)	3,892.70	607.6	93	4,494.80	817	96	4,840.10	990.6	92.8	24.3	63.0	-0.2
201	Lentils	3,225.10	33.4	1.3	4,491.10	80.9	1.8	7,590.80	275.6	3.7	135.4	725.1	184.6
195	Cow peas (dry)	5,332.30	24.1		8,357.10	23.8		7,407.90	26.8		38.9	11.2	
197	Pigeon peas	3,602.60			3,973.20			6,807.60			89.0		
211	Pulses, nes	3,215.30	736.3		4,209.70	1,017.30	4.5	4,233.00	842.6	4.9	31.7	14.4	8.9*
210	Lupins	778	192.8	5	1,325.80	228.8	4.7	1,611.00	442.5	4.7	107.1	129.5	-6.0
205	Vetches	981.1	494.6	8	945	343.4	8.6	920.5	415	9.1	-6.2	-16.1	13.8
203	Bambara beans	105.6			162			179.5			70.0		
	Total pulses	61,666.90	5,157.60	166.30	74,491.40	6,425.10	162.60	95,978.50	11,742.60	208.40	55.6	127.7	25.3

<sup>a</sup>176: *Phaseolus vulgaris*, *P. lunatus*, *P. angularis* or *Vigna angularis*, *P. aureus*, *P. mungo* or *Vigna mungo*, *P. coccineus*, *P. calcaratus*, *P. aconitifolius*, *P. acutifolius*, *V. radiata*, *V. aconitifolia*; 187: *Pisum sativum*, *P. arvense*; 191: *Cicer arietinum*; 181: *Vicia faba* var. *equina*, *V. faba* var. *major*, *V. faba* var. *minor*; 201: *Lens esculenta*, *Ervum lens*; 195: *Vigna sinensis*, *Dolichos sinensis*; 197: *Cajanus cajan*; 211: Other pulses that are not identified separately because of their minor relevance at the international level (*Dolichos* spp., *Canavalia* spp., *Psophocarpus tetragonolobus*, *Cyamopsis tetragonoloba*, *Stizolobium* spp., *Pachyrhizus erosus*); 210: *Lupinus* spp.; 205: *Vicia sativa*; 203: *Voandzeia subterranea* or *Vigna subterranean*.

\*Diff. 2012-2017 (%).

**Table 2.** Harvested area, crop production, and estimated yearly amount of the crude protein availability for buckwheat (*Fagopyrum esculentum*), quinoa (*Chenopodium quinoa*), linseed (*Linum usitatissimum*), sesame (*Sesamum indicum*), and safflower (*Carthamus tintorius*) (FOASTAT, 2019).

Crop	2007		2012		2017		Diff. 2007-2017 (%)	
	World	Europe	World	Europe	World	Europe	World	Europe
<i>Buckwheat</i>								
Average cultivated area, 1000 ha	2,739.37	1,656.78	2,491.91	1,494.21	3,940.53	1,893.13	43.8	14.3
Average seed production, 1000 tons	2,378.85	1,472.75	2,262.98	1,318.00	3,827.75	2,045.10	60.9	38.9
Average seed yield, t/ha	0.87	0.89	0.91	0.88	0.97	1.08	11.5	21.3
Crude protein content, g/kg DM <sup>a</sup>	126	126	126.00	126.00	126	126	0	0
CP availability from the crop, 1000 tons <sup>a</sup>	272.76	168.87	259.47	151.12	438.89	234.49	60.9	38.9
<i>Quinoa</i>								
Average cultivated area, 1000 ha	76.8		172.2		173.2		125.5	
Average seed production, 1000 tons	59.1		97.4		146.7		148.2	
Average seed yield, t/ha	0.77		0.57		0.85		10.4	
Crude protein content, g/kg DM <sup>b</sup>	152		152.0		152		0	
CP availability from the crop, 1000 tons <sup>b</sup>	8.18		13.47		20.3		148.2	
<i>Linseed</i>								
Average cultivated area, 1000 ha	1,977.20	240.7	2,456.6	725.0	2,778.00	735.1	40.5	205.4
Average seed production, 1000 tons	1,658.20	201.5	2,024.7	498.7	2,794.30	799	68.5	296.5
Average seed yield, t/ha	0.84	0.84	0.82	0.69	1.01	1.09	20.2	29.8
Crude protein content, g/kg DM <sup>c</sup>	350	350	350.0	350.0	350	350	0.0	0.0
CP availability from the crop, 1000 tons <sup>c</sup>	528.1	64.2	644.9	158.8	890	254.5	68.5	296.4
<i>Sesame</i>								
Average cultivated area, 1000 ha	7,126.50	0.3	8,536.2	0.3	9,983.20	0.6	40.1	100
Average seed production, 1000 tons	3,631.00	0.2	5,406.9	0.4	5,534.90	0.6	52.4	200
Average seed yield, t/ha	0.51	0.69	0.63	1.13	0.55	1.05	7.8	52.2
Crude protein content, g/kg DM <sup>d</sup>	255	255	255.0	255.0	255	255	0	0
Average CP recovery from meal, 1000 tons <sup>d</sup>	842.6	0	1,254.7	0.1	1,284.40	0.1	52.4	0*
<i>Safflower</i>								
Average cultivated area, 1000 ha	753.9	0.4	968.6	16.4	840.8	154.6	11.5	38550
Average seed production, 1000 tons	617.5	0.2	606.9	9.1	741.5	105.9	20.1	52850
Average seed yield, t/ha	0.74	0.48	0.80	0.56	0.83	0.69	12.2	43.8
Crude protein content, g/kg DM <sup>e</sup>	211	211	211.0	211.0	211	211	0	0
Average CP recovery from meal, 1000 tons <sup>e</sup>	118.6	0	116.5	1.7	142.4	20.3	20.1	1060.4*

CP: crude protein; DM: dry matter

<sup>a</sup>Air dry basis (Farrel, 1978)<sup>b</sup>Data on Dry Matter basis and Crude Protein from De Bruin (1964)<sup>c</sup>Adopting a Dry Matter (DM) content of 906 g/kg (Heuzé et al., 2017a) and a minimum Crude Protein (CP) content of 350 g/kg DM (Sammour, 1999)<sup>d</sup>Bahkali, Hussain, Basahy (1998).<sup>e</sup>Adopting an average dry matter content of 910 g/kg and an average crude protein (CP) content of 211 g/kg DM (Heuzé et al., 2015)

\*Diff. (%) 2012-2017.

control were observed as far as growing performances and slaughter yields. Trypsin and chymotrypsin inhibitor contents differ between genotypes and may negatively affect the nutritional value of this pulse for monogastric species (Bampidis and Christodoulou 2011) though heat treatments can mitigate the impairment of nutrients absorption. More in general, grain processing can improve the nutritive quality of chickpeas used in pig feeding. Christodoulou et al. (2005) showed that substituting partially or totally the SBM (42.4% CP on DM) with extruded chickpea (23.9% CP as fed) up to 300 g/kg in diets for growing pigs did not result in a significant difference in terms of body weight gain, FCR and carcass yields, nor they were able to find any significant treatment-to-sex interaction. In comparison with processed chickpea, raw grains (100 g/kg; 22.9% CP as fed) gave the poorest results in terms of body weight gain and daily feed consumption (Christodoulou et al. 2005). In the diet for young turkeys (1–70 days of age), raw chickpea seeds (22.9% CP on DM) up to 240 g/kg have been tested as a partial substitute to SBM (Ciurescu et al. 2020). These

authors did not report significant differences among experimental groups as far as the feed intake, FCR, carcass, breast, and legs yields, but the bodyweight gain resulted significantly increased up to 5.94 kg (vs. the value of 5.76 kg in the control group) in chickpea fed birds for 70 days. Chickpea are available as Desi and Kabuli varieties, the latter one being richer in starch and with lower fibre content. Desi and Kabuli chickpeas, tested as protein sources in farmed fish diets, showed high CP apparent digestibility for Nile tilapia (0.882), but ranked lower (0.906–0.911) in comparison with other grain legumes such as pea and white lupin when fed to rainbow trout at 300 g/kg in the diet (Magalhães et al. 2018). Protein pea (*P. sativum*) is particularly suitable for feed formulation due to its amino acid availability, low level of anti-nutritional factors and low tegument/endosperm ratio (Pulse Australia 2017); for these reasons, it is one of the legumes of choice in the feed industry. The well balanced amino-acid profile is limited by a deficiency in methionine and other sulphur amino acids even though the protein is particularly rich in arginine

(Spielmann et al. 2008). Tannins and trypsin inhibitors can impair the nutritional value of pea, but they have been much reduced by genetic selection and are at a very low level in current varieties (Mihailovic et al. 2005). Standardised ileal digestibility of pea proteins in pigs is about 82%, but the ileal digestibility of lysin is somewhat higher than the threshold of 85% (Mihailovic et al. 2005). Compared to soybean protein isolate and SMB, the standardised ileal digestibility of lysin (96%) and threonine (88%) of pea protein isolate in pigs did not differ and no differences in total tract CP digestibility (96%) were found (Mathai et al. 2017). In poultry, the digestibility of pea protein is quite high reaching the 84% threshold (Mihailovic et al. 2005), and the addition of peameal in diets for broilers can improve the carcass quality and the lipid profile, without adverse effects on growth performance (Laudadio and Tufarelli 2010). The replacement of 20% of SBM protein with pea protein increased the beta and gamma globulin contents of boiler blood suggesting potentially interesting effects on the birds' immunity (Bingol et al. 2016). The digestibility of field pea and other pulses (Desi and Kabuli chickpeas, faba bean and white lupin) as alternative protein sources have been tested in rainbow trout and Nile tilapia by Magalhães et al. (2018). At the protein pea (24.9% CP on DM) inclusion level of 300 g/kg in the diet for rainbow trout, the apparent digestibility coefficients for the dry matter, crude protein, and gross energy were the highest (0.773, 0.933 and 0.819, respectively). In Nile tilapia, only the CP apparent digestibility (0.879) resulted higher than in the other legume grains. The lupins (blue, *L. angustifolius*; white, *L. albus*; yellow, *L. luteus*) have CP contents ranging from 28.9% to 38.3% with a lipid content spanning the 5.4–9.4% range on DM basis (Pulse Australia 2017). However, the rather low levels of methionine and lysine partially impair their biological value. In addition, they have teguments typically rich in cellulose and moderate amounts of polyphenolics (Ronchi et al. 2010). For these reasons, they have limited nutritional value for monogastric, especially for poultry. Due to such limitation, dehulling sounds are the prime seed treatment of choice for including lupins in feed for non-ruminant farmed animals and fish. A linear relationship was observed for the apparent digestibility of dry matter, gross energy, and crude protein with increasing dehulled blue lupin meal (49.2% CP on DM) inclusion up to 30% in feed for rainbow trout (Glencross et al. 2007). Lupin hulls, that represent about 15–30% of the seed weight, can be also effectively used for ruminants and for other farmed animals. Due to the low lignin content (Brillouet and Riochet 1983), the use of lupin hulls is well established as a feedstuff for ruminant species

(White et al. 2007; Bramley et al. 2012). However, lupin hulls can be used in the place of other by-products in pseudo-ruminant species. A diet including 50 g/kg of white lupin hulls (78.1% Neutral Detergent Fibre, NDF and 63.9% Acid Detergent Fibre, ADF, as fed) and the same amount of barley has shown to substitute wheat bran (100 g/kg) of a control diet for growing rabbits (from 31 to 71 days of age) with no adverse effects on the average growth rate (52.8 g/day), average daily feed intake (157.5 g/day) and feed conversion ratio, FCR (2.99) (Volek et al. 2013). The faba bean (*Vicia faba* var. minon) has a lower nutrient content than the different varieties of lupine and more fibre than a pea. However, the high digestibility of its components means that its metabolisable energy for pigs, chickens and ruminants is alike to that of peas and lupins (Pulse Australia 2017). Despite the interesting compositional values of faba bean, a total substitution of SBM with it in diets for slow-growing chickens reared under the organic method did not give bird survivorship and growing results comparable to those obtained with SBM, suggesting that only a partial substitution should be considered for chicken, especially in the first rearing period (i.e. 1–60 days) (Dal Bosco et al. 2013). Except for a lower fat content and slightly higher pH value of the breast, no differences were recorded by these authors for chemical and technological properties of chicken breast and drumstick. Also for laying hens diets, faba beans, raw (30.2% CP on DM) or expander-processed (28.5% CP on DM) in substitution of SBM (53.8% CP on DM) at 100 g/kg inclusion levels, gave no comparable results, but at a lower inclusion level (50 g/kg diet) faba beans can be considered acceptable for the egg production rate, egg mass production, feed consumption and FCR (Koivunen et al. 2014). Partial replacement of SBM (50.6% CP on DM) with fermented or raw faba beans (28.2 and 27.4% CP on DM) did not improve the growth performance of young turkeys in an 8-weeks feeding trial (Dražbo et al. 2018). Similar results were reported also in pigs (Partanen et al. 2003) and the limitation of the apparent ileal digestible threonine and tryptophane in faba bean including diets and/or the occurrence of vicine and convicine (Dal Bosco et al. 2013) have been evoked as the major causes of poor growth results. As for the lupins and pea, the removal of the tegument significantly improves the nutritive value of these pulses for monogastric, and the integuments can be used in ruminant diets. Total substitution of SBM (48% CP, 78.3 g/kg diet) with dehulled/micronized faba bean (36% CP, 130 g/kg diet) has been tested by Tufarelli and Laudadio (2015) in a 12-weeks growing trials on

120-day-old guinea fowls with promising results concerning growing performance, yield and compositional traits of fowl meat.

Minor pulses such as the common vetch (*Vicia sativa*) as well as the bitter vetch (*V. narbonensis*), the red peas (*Lathyrus cicera* and *L. sativus*) and the ervil (*Vicia ervilia*) do not adapt to the feeding of monogastric due to their poor palatability and the content of anti-nutritional compounds but are considered alike to the pea for feeding the ruminants. Overall, their availability is very limited and detailed statistics are lacking. However, the common vetch grains are less costly (in comparison with alternatives) and rich sources of protein and minerals for farmed animals, are of high digestibility and have a high energy content, and can be used to partially or totally replace soybean meal (Huang et al. 2017). In dairy cows, wethers, and goats the apparent digestibility of common vetch included in the diets at about 290 g/kg was 0.81 (Guedes and Dias da Silva 1996), 0.753 (González and Andrés 2003) and 0.730 (Hadjipanayiotou et al. 1985), respectively. Surprisingly, common vetch, included at 297 g/kg in the diet for juveniles of silver perch, reached a rather high crude protein apparent digestibility (0.848; Booth et al. 2000). Collins et al. (2005) showed uncommon results by feeding common vetch (var. Morava) to growing pigs between 91 and 161 days of age. These authors noted that when pigs were fed on a diet including from 0 to 225 g/kg of common vetch (27.5% CP as fed), no difference was observed for body weight, growth rate, feed intake, and FCR up to the 119th day of age. Decreasing relationships were then observed for inclusion level passing from 0 to 225 g/kg diet possibly due to a buildup of cyanoalanine toxin related to the increasing feed intake of older pigs. These evidence suggest that vetch can be administered to growing pigs at a higher inclusion level (i.e. 225 g/kg diet) to younger animals than the older ones (i.e. more than 119 days of age), for which the inclusion level should be no more than 150 g/kg.

Other protein-rich promising plants are the pseudo-cereals such as amaranth (*Amaranthus* spp.), quinoa (*Chenopodium quinoa*) and buckwheat (*Fagopyrum esculentum*), seeds that are relatively unimportant on a global scale, can be significant contributors to the human diet in certain regions (Fletcher 2016). They are a good source of polyunsaturated fatty acids (PUFA) (Alvarez-Jubete et al. 2009), particularly quinoa which contains high linolenic acid (8.3%), and are also rich in minerals, with Mg and Ca content, ranging from 203.4 to 279.2 mg/100g DM and from 32.9 to 180.1 mg/100g DM, respectively (Alvarez-Jubete et al. 2009). A 100 days

trial with diets containing 10% amaranth (foliage, raw or heat-treated grains) (172.4–173.8 g/kg as CP) fed to Large White × Landrace pigs for 100 days in comparison with a control diet including meat-and-bone meal (183.2 g/kg as CP) was performed by Zralý et al. (2004). Better daily body weight gain (0.78 kg/day) and FCR (2.45) were reported by these authors in pigs fed raw or heat-treated amaranth grains, respectively, than amaranth foliage or animal proteins with no differences as far as the clinical status and biochemical analysis of blood plasma. It can be also expected that the high contents of lipids, essential fatty acids, particularly linoleic acid and squalene may be effective in wholesome pork production by modification of fatty acid composition (Zralý et al. 2004). Good results for amaranth as a substitute for animal protein (fish meal, FM) have been obtained also in poultry. Pisarikova et al. (2012) assessed the effect of amaranth raw grains (16.6% CP as fed; 8% in the diet), heat-processed grains (17.3% CP as fed, 8% in the diet) and dried foliage (11.3% CP as fed, 3% in the diet) in male and female broiler performance, carcass characteristic and meat quality in comparison with the control diet including 3% of FM (64% CP, as fed). After 42 days, no differences were recorded for meat chemical composition, live weight and FCR. Minor adverse effects on the male and female live weight were found for diets including raw grains or amaranth dried foliage, suggesting that heat-treatment can be a suitable option for the use of amaranth grains as a substitute of FM. Contrasting findings on the amaranth inclusion in chickens' feed with respect to the cholesterol-lowering effect has been reported in the literature (Popiela et al. 2013; Longato et al. 2017), but a reducing effect on the meat lipoperoxides for amaranth inclusion up to 10% in broiler diet was observed by Longato et al. (2017).

Food industry by-products such as the dried distiller grains with soluble (DDGS) are a good source of protein. Industrial processes, such as the one of starch extraction from starchy vegetables (e.g. potato) and the process for the production of bioethanol, can generate aqueous flows that contain good amounts of protein (27–40%) (U.S. Grains Council 2018). However, the values data on the chemical composition and nutritional value of DDGS are highly variable depending on the raw materials and the manufacturing process (Spiehs et al. 2002; U.S. Grains Council 2018). Particularly DDGS wheat contains the highest crude protein percentage (40.67%) while DDGS maize the lowest (27.2%), an intermediate crude protein percentage was found in DDGS sorghum (31.5%) (U.S. Grains Council 2018). Research carried out in recent years has

demonstrated the possibilities of corn DDG as feed for beef and dairy cattle, sheep, swine and poultry due to its interesting content of crude protein (25–30% on DM), high calorific value and mineral content (i.e. 0.43–0.83% on DM as phosphorus) (Pecka-Kiełb et al. 2017).

Several oil-bearing plant species are commonly used worldwide to obtain vegetable oils. Among them, linseed (*Linum usitatissimum*), sesame (*Sesamum indicum*) and safflower (*Cartamus tintorius*) are gaining interest due to the technological and functional traits of their oils. The derived partially defatted by-products, such as meals, cakes, and expellers, are rich in crude proteins (Table 3). Linseed meal is a good source of proteins and of nitrogen-free extracts as well, with a low content of ash. Sesame meal is another excellent protein source with low crude fibre content, especially for partially dehulled seeds. In particular, sesame meal contains high levels of methionine, cystine and tryptophan, even though it is rather deficient in lysine (Ravindran and Blair 1992). In the ration for Awassy ewes up to 15% of the

diet DM, sesame meal was tested by Obeidat et al. (2019) in substitution of the same quota of SBM. These authors reported no difference for dry matter, crude protein and fibre intakes and digestibility but increased fat intake and digestibility was observed. An increase milk production was also observed comparing sesame meal vs. SBM fed ewes (1.27 vs. 1.00 kg/day, respectively) along with an increased fat and protein yields and a better feed-to-milk conversion (1.64 vs. 2.15, for 15% sesame meal and 15% SBM in the diet, respectively). The level of proteins in safflower meal strongly depends on the hulls in the meal and the processing methods (Hertrampf and Piedad-Pasqual 2000) (Table 3).

Protein from plant leaves is gaining interest as a protein source for human and non-ruminant livestock. They are mainly composed of an enzymatic complex called RUBISCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) which plays a crucial role in the photosynthetic process. RUBISCO is in the stroma of the chloroplasts and can amount to 50% of the total

**Table 3.** Proximate composition (% DM), energy (kcal/kg DM), other constituents, and amino acids content in oil extraction processed linseed (*Linum usitatissimum*), sesame (*Sesamum indicum*) and safflower (*Cartamus tintorius*).

	Linseed meal/expeller	Sesame meal		Safflower meal/expeller	
		Whole	Part. dehulled	Whole	Part. dehulled
Proximate composition					
Dry matter, %	90.00 <sup>a</sup>	92.40 <sup>a</sup>		91.30 <sup>a</sup>	91.2 <sup>a</sup>
Crude protein	34.00 <sup>b</sup> –35.00 <sup>a</sup>	45.00 <sup>a</sup>	40.00 <sup>b</sup>	23.00 <sup>a</sup>	40.00 <sup>b</sup> –43.11
Fat	2.00 <sup>a</sup> –6.02	4.80 <sup>a</sup>	6.00 <sup>b</sup>	1.40 <sup>a</sup>	1.60 <sup>a</sup> –6.02
Ash	6.20 <sup>a</sup> –8.02	13.00 <sup>a</sup>	12.00 <sup>b</sup>	4.70 <sup>a</sup>	6.00 <sup>b</sup> –7.11
Crude Fibre	9.00 <sup>b</sup> –9.21	6.70 <sup>a</sup>	8.00 <sup>b</sup>	31.40 <sup>a</sup>	11.51 <sup>a</sup> –15.02
NFE	37.60 <sup>a</sup> –43.02	24.20 <sup>a</sup>	34.00 <sup>b</sup>	30.80 <sup>a</sup>	28.00 <sup>a</sup> –33.02
Energy					
DE – cattle	15.00–16.23	16.60 <sup>d</sup>			
ME – cattle	11.60–12.63	12.50 <sup>d</sup>		8.30 <sup>e</sup>	11.70 <sup>e</sup>
DE – pig		13.10 <sup>a</sup> –15.74		8.1 <sup>e</sup> –11.11	14.70 <sup>a</sup>
ME – pig	14.30 <sup>c</sup>	14.40 <sup>d</sup>			
DE – chicken	13.50 <sup>a</sup>	14.40–14.71		6.70 <sup>a</sup>	11.60 <sup>a</sup>
ME – chicken	6.70 <sup>b</sup> –7.11	9.50–9.91	8.36 <sup>b</sup>	5.20 <sup>f</sup> –5.41	7.50–8.42
ME – hens	–			8.96	
DE – rabbits	14.10 <sup>a</sup>				
DE – fish (rainbow trout)	17.70 <sup>a</sup>				
ME – fish (rainbow trout)	13.10 <sup>a</sup>	12.80 <sup>c</sup>			
Other constituents					
Ca, g/kg DM	3.80 <sup>a</sup> –4.02		20.00 <sup>b</sup> –23.31		3.00 <sup>b</sup>
P, g/kg DM	8.00 <sup>b</sup> –8.51		12.90 <sup>a</sup> –13.02		8.00 <sup>b</sup>
Phytic acid, g/kg DM			36.0 <sup>b</sup>		
Phytate P, g/kg DM			1.8 <sup>b</sup>		
Amino acids, g/100 g N <sup>a</sup> or g/100 g CP <sup>b</sup>					
Arg	3.10 <sup>a</sup> –9.82	4.80 <sup>a</sup> –12.82		2.00 <sup>a</sup>	3.40 <sup>a</sup> –9.22
Cys	2.00 <sup>b</sup>	2.20 <sup>b</sup>			1.60 <sup>b</sup>
Gly	5.70 <sup>b</sup>	1.10 <sup>a</sup> –5.62			3.00 <sup>b</sup>
Hys	0.80 <sup>a</sup> –2.22	2.8 <sup>b</sup>		0.50 <sup>a</sup>	1.00 <sup>a</sup> –2.52
Ile	1.70–4.52	1.90 <sup>a</sup> –3.82		0.50 <sup>a</sup>	1.50 <sup>a</sup> –4.22
Leu	2.20 <sup>a</sup> –6.02	3.10 <sup>a</sup> –7.62		1.30 <sup>a</sup>	2.40 <sup>a</sup> –6.22
Lys	1.20 <sup>a</sup> –3.02	1.30 <sup>a</sup> –3.82		0.70 <sup>a</sup>	1.20 <sup>a</sup> –3.22
Met	0.60 <sup>a</sup> –1.92	1.30 <sup>a</sup> –3.22		0.30 <sup>a</sup>	0.60 <sup>a</sup> –1.62
Phe	1.60 <sup>a</sup> –4.82	2.10 <sup>a</sup> –5.52		1.00 <sup>a</sup>	1.80 <sup>a</sup> –5.02
Thr	1.30 <sup>a</sup> –4.02	1.60 <sup>a</sup> –3.82		0.60 <sup>a</sup>	1.30 <sup>a</sup> –3.32
Trp	1.80 <sup>b</sup>	0.70 <sup>a</sup> –1.52		0.20 <sup>a</sup>	0.50 <sup>a</sup> –1.52
Tyr	2.90 <sup>b</sup>	4.5 <sup>b</sup>			3.00 <sup>b</sup>
Val	1.80 <sup>a</sup> –5.42	2.20 <sup>a</sup> –4.82		1.10 <sup>a</sup>	2.00 <sup>a</sup> –5.52

DM: dry matter; NFE: nitrogen free extracts; DE: digestible energy; ME: metabolisable energy; CP: crude protein.

<sup>a</sup>Data from Petersen et al. (1976); <sup>b</sup>Data from Ravindran and Blair (1992); <sup>c</sup>Data from Hertrampf and Piedad-Pasqual (2000); <sup>d</sup>Data from Heuzé, Tran, Nozière, et al. (2017); <sup>e</sup>Data from Heuzé, Tran, Bastianelli, et al. (2017); <sup>f</sup>Data from Heuzé et al. (2015).



leaf protein. The composition of RUBISCO is quite constant through various green leaf plants and represents a rich source of essential amino acids (Edelman and Colt 2016). Even though the high fibre content of these potential sources would limit their use in monogastric diets, discouraging any practical perspective in the feed industry, to date there are several techniques for the physical separation of the protein fraction from the fibrous ones, such as mechanic pressing, water extraction, leaf/stem separation, as well as other procedures can help to increase the protein recovery (steam injection, acid precipitation, ultra-filtration and spray drying) for practical applications (Bals et al. 2012). Leaf protein concentrates from red clover (*Trifolium pratense*) and Italian ryegrass (*Lolium multiflorum*) (Szymczyk et al. 1996) and from gliricidia (*Glyricidia sepium*) were tested in chicken diets in comparison with SBM and FM. Red clover (defatted, 60.9% CP on DM basis) and Italian ryegrass proteins (50.5% CP on DM basis) mixed in a 1:1 ratio with SBM (protein basis) gave similar results to SBM alone (400 g/kg) on the total live body weight gain of 0–4 weeks (757–798 g) and 5–8 weeks (1143–1450 g) chicks. Interestingly, all the leaf protein concentrates and red clover (unextracted) increased the Vitamin A (up to 248.7 µg/g, wet basis) and carotene (20 µg/g, wet basis) contents in liver, in comparison with SBM fed chickens (117.6 mg/kg and below detection level, for Vitamin A and carotene, respectively) (Szymczyk et al. 1996). Iso-protein replacement of FM protein with gliricidia leaf protein concentrate was evaluated in diets for broilers by Agbede and Aletor (2003). Weight gain, average feed consumption, and feed efficiency declined as the level of leaf protein increased (from 1.81 to 7.24%) at the expense of FM protein but the nitrogen retention of chicks fed on 1.81–5.43% leaf proteins based diets was similar to those of chicks fed FM diet. Growing performance, carcass traits and haematological indices indicated that FM can be partially substituted (up to 25%) by gliricidia leaf proteins concentrates with no adverse effect on rearing performance and product quality of broilers (Agbede and Aletor 2003). Four isonitrogenous and isoenergetic diets for sharp-snout sea bream (*Diplodus puntazzo*) containing increasing levels (0, 7, 14, and 21%) of alfalfa protein concentrate (52–55% CP) partially substituting FM were tested by Chatzifotis et al. (2006). The increasing inclusion of leaf proteins did not affect the fish survival nor impact on the daily feed intake, hepatosomatic index or visceral index. The 14% inclusion did not differ from the control as far as the FCR (1.45 vs 1.44, respectively), but the weight gain of fish

fed leaf protein resulted impaired in comparison to the control. Partially contrasting results were obtained in tilapia by Olvera-Novoa et al. (1990) who tested diets in which FM (66.6% CP as fed, 500 g/kg in the diet) was replaced from 15 to 55% by alfalfa leaf (cytoplasmatic) protein concentrates (69.2% CP, as fed). These authors concluded that alfalfa cytoplasmatic proteins replacing up to 35% of FM gave better results in terms of growth rate than the 50% FM fed tilapia.

## SWOT analysis

### Strengths

Some seeds have been considered as novel attractive products for animal nutrition, particularly during stressful events, in relation to the positive effects on the immune responses, and on the improvement of the growth performance. Whole linseed supplementation in the diet of periparturient ewes or cows can modulate immune reactivity by influencing cytokine production (Caroprese et al. 2015; Didara et al. 2015). In the dairy cow, supplementation with linseed can increase milk yield and improve the fatty acids profile increasing the PUFA fraction (Caroprese et al. 2010). Dietary inclusion of linseed in monogastric animals is very effective in increasing the PUFA fraction (mainly the FA of the n-3 series) in food-derived products. An example is the doubling of n-3 FA in rabbit meat after an inclusion level of extruded linseed. Also, quinoa seeds contain important physiological functionalities, among which anti-microbial and anti-inflammatory activities. Particularly, Marino et al. (2018) found that quinoa seeds supplementation can help lamb to cope with stressful events due to the close link between stress responses and the immune system and for improving meat tenderness. Under an agro-ecological perspective, another strength point is that the use of protein crops has also environmental advantages because they fix nitrogen in soils reducing the use of fertiliser. In addition, the use of by-products as an alternative protein source could be an environmentally sustainable practice for cattle husbandry. McGinn et al. (2009) showed that the use of corn distillers can reduce enteric methane loss from beef cattle. Finally, the availability of new protein sources is crucial to meet the growing demand both as food and for the formulation of livestock feed.

### Weaknesses

Legume grains contain anti-nutritional factors (ANFs) such as digestive enzyme inhibitors, lectins, tannins and phytic acid which reduce the bioavailability of nutrients, and saponins that increase intestinal

permeability, allowing toxins and gut bacteria to interact with the immune system (Rebello et al. 2014). The tegument of lupins is rich in cellulose and therefore they have limited nutritional value for monogastric, especially for poultry, but represent a source of digestible fibre for ruminants. For this reason, the removal of the tegument is recommended to improve the degree of utilisation of the lupine by monogastric species. The broad bean has a lower nutrient content than the different varieties of lupine and more raw fibre than the pea. Chickpea grains can represent an alternative protein source to soybean meal and to energy concentrates in animal feeding, thus, their nutritional value can help to increase the sustainability of livestock systems. However, its excellent nutritional characteristics are accompanied by the occurrence of some bioactive substances with anti-nutritional effects (Singh 1988). Hence, research on methods for the reduction of chickpeas ANFs is ongoing. As an example, it has been shown that agronomic practices such as the right choice of sowing time (i.e. winter sowing against spring sowing) and high seeding rate (i.e. high density, 110 seeds  $m^{-2}$  vs. low density, 70 seeds  $m^{-2}$ ) can reduce trypsin inhibitors (−4%, on average) and/or  $\alpha$ -galactosides (−9%, on average) in two cultivars of Kabuli chickpea (Primi et al. 2019). On the other hand, post-harvest treatments can play a relevant role in lowering the ANFs content of grain legumes. With special regard to the minerals, despite the content in some protein sources, a not negligible quota of them can be in the phytate form that reduces the bioavailability for monogastric animals. Phytase can be added to the diets for pigs (i.e. 0.1 g/kg), with promising results in terms of calcium and phosphorous digestibility (Reis de Souza et al. 2017). Similarly, 0.2 g/kg diet of phytase added to chickpea-containing diets for turkeys exerted some effects on the P and Fe plasma levels and resulted beneficial for the tibia mineralisation (Ciurescu et al. 2020). However, the market price of chickpea, as well as that of lentils (*Lens culinaris*), is generally higher than the price of other grain feeds because it is mainly intended for human consumption. As far as the leaf protein concentrates, the concentration methods could increase the possibilities of use of these ingredients in animal nutrition, but to date further developments are still needed to make these processes suitable in practice also from the point of view of the economic convenience (Bals et al. 2012).

### Opportunities

The use of alternative proteins source in livestock nutrition is an opportunity linked to the current

growing food protein demand and feed-food competition. Particularly, alternative protein sources can be a valuable tool in lowering feed cost in animal production and could offer a possibility for the development of a specific market segment in the EU. In addition, the 'alternative' protein crops can adapt to pedoclimatic conditions different from the place of origin. Some protein-rich plants harvested mainly as grains, such as the safflower, are showing interesting traits also as protein-rich fodders (Landau et al. 2004; Danieli et al. 2011). These minor and often termed neglected crops are often characterised by good plasticity to environmental change that makes them extremely attractive in defying new production systems. In the light of increasing the environmental sustainability of the animal farming systems, some pieces of evidence suggest that bioactive compounds, such as the polyphenolic components of the legumes, can help in reducing the emission of methane from rumen fermentation (Guglielmelli et al. 2011; Calabrò et al. 2012).

### Threats

Factors that could limit the expansion of the use of alternative protein crops are linked to (1) the price unpredictability and the profitability of competition cultivation and (2) the lack of planned strategies by rural development programs to support protein crops. A factor that can preclude a wide utilisation of chickpea as an alternative protein source in animal feeding is the low yield, often worsen by outbreaks of *Ascochyta* blight, a necrotic disease due to the fungus *Ascochyta rabiei*. Research is ongoing to study the effect of seeding practices (e.g. sowing date, seeding rate) on the yield, as well as on the nutritive value of different cultivars of chickpeas, but data acquired on the field (Ruggeri et al. 2017) suggest the anticipated sowing (in winter) as the best choice in the Mediterranean area to maximise yield. Future investments in genetic and agronomic research could help the diffusion of these grain legumes and other protein-rich plants as animal feeding crops. Proteins from DDGS can deliver to the animal's undesired biogenic substances, such as mycotoxins if bioethanol is produced from low-quality grains. Positivity from 80% up to 100% of samples to zearalenone and deoxynivalenol, two of the most representative *Fusarium*-toxins, was reported in DDGS sampled in North America and Asia (Rodrigues and Naehrer 2012). For this reason, it is highly recommendable that DDGS batches are examined in reference laboratories for the presence of mycotoxins before using (Pecka-Kiełb et al. 2017).

## Future perspectives

There is a great demand for protein-rich feedstuffs in Europe with a deficit of about 70% high-protein materials (Houdijk et al. 2013; Bouxin 2014), covered in large part by imported soybean and soy-derived products. On the other hand, the cost-effectiveness of soybean meal, that pushed its use in the formulation of the diet of farm animals since 1960, is progressively reduced because the price of this commodity constantly rose and today forecasts go in the same direction (The World Bank 2020). These facts should ensure good opportunities for the development of an integrated farming system focussed on coupling livestock farming to the cultivation of cereals and protein-rich plants such as pulses, in rotation management schemes (Watson et al. 2017). In addition, some recent studies have shown that the environmental impact of replacing soybean meal in livestock diets with on locally cultivated pulses (Baumgartner et al. 2008; Nemecek et al. 2008) and/or other protein-rich plant-derived products (Sasu-Boakye et al. 2014) could be mitigated. However, if the locally cultivated protein-rich plants will become a real scenario for animal farming in the next future, some main changes will have to take place at the European level (Watson et al. 2017): (i) increasing grain/protein yield and yield stability, including varietal selection for tolerance to drought and plant diseases; (ii) better assessment of the economic relevance of pulses and other protein-rich plant feedstuffs; (iii) the valorisation of the product, giving adequate economic stimulus for farmers to invest in protein-rich plants cultivation, including public policy support; (iv) gaining market advantages from the environmental improvements. In some of these aspects, the scientific research (plant research and breeding, agronomics, feedstuff processing, animal feeding and nutrition) can give a valuable contribution towards more sustainable animal production systems. As a real example, recent Italian research has shown how suitable agronomic strategies can improve the protein content and nutritional value for pulses (Primi et al. 2019) as well as for cereals (Rossini et al. 2018) cultivated in the Mediterranean region.

## Microbial biomasses

### General aspects and availability of microbial biomasses

The name Single Cell Protein (SCP) was coined for yeasts, fungi, bacteria and microalgae to describe the protein production from biomass, originating from

different microbial sources. However, this term is limited in the case of microalgae since they are characterised by a broad *spectrum* of other compounds of relevant importance from a nutritive point of view (peptides, carbohydrates, lipids, vitamins, pigments, macro and microminerals) (Becker 2007).

Microalgae are microscopic unicellular organisms capable to convert solar energy to chemical energy via photosynthesis, although few species are heterotrophic. Microalgae are classified into diatoms (Bacillariophyceae), green algae (Chlorophyceae) and golden algae (Chrysophyceae) that are eukaryotic, and blue-green algae cyanobacteria (Cyanophyceae), that are prokaryotic. The most important phototrophic species belong to the genera *Arthrospira*, *Chlorella*, *Dunaliella* and *Haematococcus*. They can be used to produce a wide range of biomolecules (astaxanthin, lutein,  $\beta$  carotene, chlorophyll, phycobiliprotein, PUFA,  $\beta$ -1,3-glucans, and pharmaceutical and nutraceutical compounds) and are involved in several industrial applications, as feed for livestock, poultry and farmed finfish species. According to EU Regulation, the microalgae registered as animal feed or ingredients for animal feed are *Spirulina maxima* and *Spirulina platensis* and the species of the genus *Schizochytrium*. The microalgae can be added to the feed as a whole or defatted algal meal, microalgae-based oils, or dried or freeze-dried algae biomass. Microalgae have several uses in aquaculture, for feeding molluscs, echinoderms, and crustacean larvae as well as for the direct feeding of the larval stage of some fish species or for feeding live prey (rotifers) to fish larvae (Borowitzka 1998). The criteria of choice are highly selective, and this is the reason of the restricted number of species that are utilised in the aquaculture sector (Muller-Feuga 2000) (gen. *Chlorella*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema*, *Thalassiosira*, *Haematococcus* and *Tetraselmis*) due to the characteristics that should be met: ease of culture, lack of toxicity, nutritional value, cell size and shape, digestibility. Microalgae exhibit considerable metabolic plasticity and the possibility to modulate their nutritional value by the manipulation of culture conditions (such as medium nitrate concentration, CO<sub>2</sub> concentration, light intensity and the timing of harvest) represents a peculiarity that is widely exploited to modulate their chemical-nutritional properties. Other than carbohydrates, lipids and carotenoids, high amounts of protein can also accumulate and be extracted from microalgae. Protein-rich microalgae biomass has been used successfully to partially replace fishmeal in the aquaculture industry and as a

source of protein and biomolecules in livestock. Some species of microalgae can produce as many proteins (2.5–7.5 tons/Ha/year) as other rich sources of proteins, for example, egg, meat, milk, etc. (Khan et al. 2018). During the decades 1980–1990, microalgae were studied mainly for the rearing of larvae and juveniles of shellfish and finfish, as well as for feeding live preys (zooplanktons) needed for feeding fish juveniles. Recently the interest towards the utilisation of microalgae as a source of protein in total or partial replacement of fishmeal in diets for different fish species with high protein request (salmon, rainbow trout, chinook salmon) but also for less demanding species, like Nile tilapia, Indian major carp, common carp, that have the blue-green algae as common components of their diet, has increased.

The species experimented as ingredients in aquafeeds are *Arthrospira* sp., *Tetraselmis*, *Phaeodactylum tricornutum*, *Nannochloropsis* sp., *Isochrysis* sp., *Navicula* sp., *Haematococcus pluvialis*, *Nanofrustulum* sp., *Scenedesmus almeriensis*, *Chlorella vulgaris*, utilised as single species or in consortia (Rahman Shah et al. 2018). The trials performed using microalgae in partial replacement of fishmeal produced not consistent results. Walker and Berlinsky (2011) on juvenile Atlantic cod (*Gadus morhua*) tested a combination of dried *Nannochloropsis* sp. and *Isochrysis* sp. in partial replacement (15 or 30%) of fish meal protein, finding a worsening of fish performance (growth, feed intake) at the highest percentage of replacement. The use of a blend of *Tisochrysis lutea* and *Tetraselmis suecica* dried biomass replacing 15, 30 or 45% of fish meal protein in diets low in fishmeal content in European sea bass produced no effects on fish growth despite the decline in feed digestibility (Cardinaletti et al. 2018). Palatability and nutrient bioavailability is strictly dependent of the microalgal species as it is hampered by the high complexity of their cell walls, which may introduce anti-nutritional factors and may harm the intestinal tract and result in inflammation and reduced nutrient uptake in fish species. Proper processing techniques should be applied before the inclusion of microalgae biomass are considered in aquafeeds (Batista et al. 2020). In general, the nutritive value of microalgae appeared lower in comparison with combinations of fish meal and oil, but performed better than diets based on plant protein and oil sources, that are increasingly utilised as alternatives to fish meal and oils in the current commercial aquafeeds.

In the case of livestock (ruminants and monogastrics), the microalgae tested are well summarised in the recent review by Madeira et al. (2017). *Arthrospira*

(*Spirulina*) *platensis*, *Schizochytrium* sp., *Chlorella vulgaris* and *Chlorella* sp. were utilised as feed ingredients for ruminants, pigs, chicken, in percentages ranging from 0.00003 to 20%, with different effects, in relation to the inclusion levels and microalga species, the species and life stage of the animals fed but also in relation to the processing realised on the biomass. Indeed, the presence of cellulose in the microalga wall (except for cyanobacteria, like *Arthrospira* (*Spirulina*) and *Aphanizomenon*) differently affects the results in monogastric and ruminants, if microalgae are not previously submitted to an appropriate process to increase their digestibility.

The development of technologies for microalgae biomass production started sixty years ago at the Massachusetts Institute of Technology in a project to produce the green microalga *Chlorella* for human foods using closed culture systems, so-called photobioreactors (PBRs) in addition to the more traditional open pond algae cultivation systems ('high rate ponds' or HRP), consisting in circular or raceway ponds, ranging from 100L to more than 10<sup>9</sup> L. Currently, the raceway-type, paddlewheel mixed ponds (HRPs) are the dominant cultivation systems (Benemann 2013). Autotrophic microalgal cultivation of marine species in these systems can utilise non-arable lands and water resources considered unsuitable for agriculture. In the last years, there was a growing interest in microalgae production for biofuels as well as for animal feeds, but the current high production costs represent a relevant limiting factor for this latter utilisation. Even though the term 'microalgae' is utilised as a synonym for photoautotrophic, unicellular algae utilising CO<sub>2</sub> and gaining energy solely from the light, there are numerous microorganisms currently classified as microalgae that are in fact obligate heterotrophs (Droop 1974; Gladue and Maxey 1994), and others are capable of both heterotrophic and photoautotrophic metabolism, sequentially or simultaneously. Until now, only a few heterotrophic species are produced commercially, using less expensive, well-defined mineral medium and starch or sugars as carbon source. Genera that can be reared in heterotrophic condition are *Chlorella*, *Cryptocodinium*, *Nitzia*, *Prototheca* spp., *Galdieria*, *Haematococcus*, *Nannochloropsis* or *Schizochytrium* spp. Some species show very slow growth performance while others are scarcely tolerant of environmental conditions. *Schizochytrium* and *Thraustochytrium* are highly tolerant species and are both known to accumulate large quantities of lipids within their biomass (Bumbak et al. 2011).

The global market value of microalgae is estimated to be around US\$6.5 billion, out of which about US\$2.5 billion are produced by the health food sector, US\$1.5 billion by the production of DHA while US\$700 million by aquaculture. The world annual production of microalgae is approximately 7.5 million tons (Mobin and Alam 2017), which is estimated to be only 0.7% of what would actually be needed to totally replace the protein from the fish meal in aquaculture. In addition, even if extensive research into algae cultivation in order to reduce the cultivation cost and increase the productivity of microalgae has increased dramatically over the last few decades (Beal et al. 2015), the current price of microalgae is between US\$10 and US\$30 per kg, much higher than soybean meal (0.30 \$per kg), hence global production is limited to high-value niches as human supplement and nutraceutical. The qualitative assessments of the alternative protein sources based on a combination of the current-day realities and the future potential (10–20 years) of each protein source consider that the main obstacle that will need to be overcome before development, in the case of microbial biomasses, is the economics feasibility (Hua et al. 2019), that is the main objective for the next future.

### **Microbial biomasses composition**

Microalgae are dietary sources of macro and micronutrients that provide natural ingredients and supplements in animal diets in order to meet the increasing demand of protein and energy. The nutritional composition of microalgae is generally well known and has been documented in many published paper (e.g. Yaakob et al. 2014; Madeira et al. 2017; Molino et al. 2018). Particularly, the high protein content of various microalgae species is one of the main factors promoting their utilisation in feed production (Kovač et al. 2013). *Arthrospira (Spirulina) platensis* and *Chlorella vulgaris* show the highest protein content with values ranging from 42.1 to 63% and from 20 to 60.4% (w/w on dry matter), respectively, which constitutes an enormous span (Molino et al. 2018). This wide range of protein percentage, particularly in *Chlorella vulgaris*, highlights that microalgal protein content is strongly connected to the growth phase of the microalgae and it often declines after nitrogen depletion phase, with a relative increase in carbohydrate or lipid content. Therefore, the microalgal biomass has to be harvested before nitrogen depletion state when its protein content would be at the maximum (Bleakley and Hayes 2017; Khan et al. 2018). Moreover, higher protein

contents are mostly achieved in nitrogen-rich *media* (commonly  $<450 \text{ mg N L}^{-1}$ ) (Grossmann et al. 2019). On the contrary, the essential amino acid composition of microalgal proteins is rather conserved among species, and relatively unaffected by growth phase and cultivation conditions (Guedes et al. 2015). Carbohydrates are also important nutrients in microalgae because contribute to providing energy for animals and include a large share of dietary fibre that help keeping the gastrointestinal tract healthy (Gutiérrez-Salmeán et al. 2015).

Microalgae lipids are also high-value nutrients due to the high content of PUFA such as DHA, EPA,  $\gamma$ -linolenic acid (GLA) that have recognised effects on animal health (Madeira et al. 2017). In particular, *Nannochloropsis* sp. has a high amount of fatty acids, with a percentage of long-chain PUFA equal to 45.85%, making it a promising product for the  $\omega$ -3 market, with an EPA content (33.19%) similar to *Ulkenia* sp. and *Schizochytrium* sp., according to Regulation (EU) 2017/2470 on novel foods (Molino et al. 2018). *Schizochytrium* sp. is characterised by valuable DHA concentration (certain *Schizochytrium* strains might contain levels of DHA  $>94\%$  of total n-3 fatty acids) and good growth under high-density culture conditions (Martins et al. 2013). *Nannochloropsis oculata* and *Isochrysis galbana* are two additional promising candidates for large scale production of EPA and DHA, respectively, as highlighted by Aussant et al. (2018). Moreover, under particular cultivation conditions as the two-step cultivation method, a substantial enhancement of lipid production has been achieved in *Chlorella* and *Scenedesmus* (Yu et al. 2018).

Microalgae also represent a valuable source of minerals, vitamins and carotenoids like astaxanthin, lutein, tocopherols, phycobiliproteins like phycocyanin (Bumbak et al. 2011; Molino et al. 2018). Vitamins such as niacin, nicotinate, biotin, and folic acid have been found in microalgae, particularly in *Arthrospira (Spirulina) platensis* and *Chlorella* sp. (Madeira et al. 2017), the latter one being also a valuable source of cobalamin vitamin B12.

Trace elements composition of microalgae reported in the literature is highly heterogeneous for copper ( $4\text{--}1900 \text{ mg kg}^{-1}$ ), iron ( $15\text{--}6800 \text{ mg kg}^{-1}$ ), manganese ( $19\text{--}4000 \text{ mg kg}^{-1}$ ) and zinc ( $14\text{--}5500 \text{ mg kg}^{-1}$ ) while that of selenium is rather consistent (about  $1 \text{ mg kg}^{-1}$ ) (Batista et al. 2020). The health beneficial effects of Se in relation to oxidative stress conditions, immune system, viral infections, reproduction, and thyroid function have been reviewed and seafood is one of the food commodities relatively rich in this micronutrient. In general, the mineral and trace element composition

of microalgae does not appear particularly unique relative to other common terrestrial plant-based feed ingredients, except for iron (Fe). The high Fe content of many microalgae-based ingredients is due to the fact that most microalgae products generally contain the entire dried organism, including their chloroplast proteins responsible for photosynthesis, while terrestrial plant-based ingredients are generally produced from seeds which are non-photosynthetic. So as feed industries continue to search for natural sources of key nutrients and micronutrients to replace expensive chemically synthesised feedstocks, these high levels of Fe may provide a unique and highly marketable property for certain microalgae-based products.

*Arthrospira platensis* (*Spirulina*) is the most highly consumed microalgae due to its high protein content; it is an edible, filamentous, spiral-shaped cyanobacterium, formally classified as a blue-green microalga. *Arthrospira platensis* also has nutritional benefits such as anti-hypertension, renal protective, anti-hyperlipidaemic, and anti-hyperglycaemic. *Arthrospira* (*Spirulina*) contains high levels of hypocholesterolemic  $\gamma$ -linoleic acid (GLA), B-vitamins, calcium, iron and  $\beta$ -carotene (Bleakley and Hayes 2017). The protein of *Arthrospira* (*Spirulina*) has a digestibility coefficient value of 77.6% while reference protein sources such as casein and egg have a digestibility coefficient of 95.1% and 94.2% (Becker 2007). *Arthrospira* (*Spirulina*) can be included as a protein source into the diets of ruminants, pigs, poultry, and rabbits (Holman and Malau-Aduli 2013). Several studies showed the use of algae as viable alternatives to fish meal and fish oil (Gouveia et al. 1997; Palmegiano et al. 2005; Kiron et al. 2012; Tulli et al. 2012; Qiao et al. 2014; Tibaldi et al. 2015; Sarker et al. 2016; Cardinaletti et al. 2018). Over the last years, the use of microalgae and seaweed for the development of novel products, as well as for obtaining high-added value compounds, has attracted much interest from both food and pharmaceutical industries (Barba 2017).

As a further asset for SCP massive production is represented by its antibacterial properties linked to the natural aquatic environments typically filled with bacteria and viruses that can attack fish and shellfish. Bacteria and viruses can also attack single-celled microalgae, so these microorganisms have developed biochemical mechanisms for self-defence; such mechanisms involve secretion of compounds that inhibit bacterial growth or viral attachment. For instance, compounds synthesised by *Scenedesmus costatum*, and partially purified from its organic extract, exhibit antibacterial activity because of their fatty acids longer than 10 carbon atoms in chain length, which

apparently induce lysis of bacterial protoplasts that has recently been put into commerce against well-known bacterial infections that cause bovine mastitis (Zivo Bioscience 2018). Anyway, the nutraceutical benefits of their utilisation in animal feeds still need to be fully exploited.

Ruminants can utilise algal proteins more efficiently than monogastric animals. A study of Panjaitan et al. (2010) reported that 20% of the consumed *A. platensis* bypasses degradation within the rumen, allowing for increased digestion and absorption of protein and nutrients. In this study, the *Arthrospira* (*Spirulina*) was added to the drinking water, and the daily water intake increased by 24.8 g/kg. Incorporation of 200 g/day of *Arthrospira* (*Spirulina*) in cattle feed was reported to be an economically effective method of increasing animal body condition score and body weight (8.5–11%), and daily milk production (21%) (Kulpys et al. 2009). In more detail, previous data showed an increase in milk fat (between 17.6% and 25.0%), milk protein (up by 9.7%) and milk quantity associated with dietary inclusion of *Arthrospira* (*Spirulina*) supplementation (Simkus et al. 2007). Christaki et al. (2012) demonstrated that the increased milk quality was associated with decreasing saturated fatty acids, while simultaneously increasing monounsaturated fatty acids and PUFA. These results could be attributable to *Arthrospira*'s influence on microbial protein synthesis, avoidance of rumen degradation and its nutrient-rich composition. Bezerra et al. (2010) reported that lambs increased average daily gains upon consumption of 10 g of *Arthrospira* (*Spirulina*) per day. Similar results in live weight and in body condition score of lambs were observed by Holman and Malau-Aduli (2013), but variations did not reach statistical significance. Anyway, it should be pointed out that differences in the age of the lambs in the two studies the age of the lambs was different, as well as in the *Arthrospira* (*Spirulina*) suspensions in water used to deliver it make the results not easy to be compared.

In pig nutrition, results about the supplementation of *Arthrospira* (*Spirulina*) to diets are inconsistent. Hugh et al. (1985) found growth rates of up to 9% higher in weaning pigs than their unsupplemented peers; Grinstead et al. (2000) found no growth difference between *Arthrospira* (*Spirulina*) - supplemented and unsupplemented pigs. Supplementation to pellets was reported to decrease average daily gain, whereas incorporation of *Arthrospira* (*Spirulina*) to meal diets actually increased average daily gain (Grinstead et al. 2000). Addition of *Arthrospira* (*Spirulina*) to the diet

has also been suggested to improve fertility in pigs, increasing sperm motility and storage viability (Granaci 2007).

*Arthrospira (Spirulina)* sp. is widely used in poultry nutrition and the impact of its inclusion on chicken growth and growth rates depends on the feed ingredient it replaces in the ration and on the inclusion percentage. Toyomizu et al. (2001) have shown that dietary *Arthrospira (Spirulina)* levels of 50–100 g/kg of feed ration maintain typical growth rates that are impaired at inclusion level higher than 200 g/kg. Feeding cockerel chicks, male broiler chicks, and Japanese quails with varying concentrations of *Arthrospira (Spirulina)* slightly delayed growth rates but did not affect the final growth at concentrations less than 10% (Ross and Dominy 1990). Furthermore, this study also reported that the dietary supplementation of *Arthrospira (Spirulina)* results in increased fertility rates, as well as the intensity of the egg-yolk colour. These results have been confirmed by several other studies, indicating that the inclusion of *Arthrospira (Spirulina)* at a concentration of 20–25 g/kg in the feed intensifies egg yolk colour making it more attractive for consumers (Sujatha and Narahari 2011; Mariey et al. 2012). The same result was obtained for egg yolk colour by Saxena et al. (1983) utilising *Arthrospira (Spirulina)* at levels of 3–9% of the total ration in the diet for White Leghorn layer hens. *Arthrospira (Spirulina)* effect on yolk colour results from its high-level content of zeaxanthin, xanthophylls and other carotenoid pigments, particularly  $\beta$ -carotene, which accumulate within the lipid fraction of the yolk. Such an effect has also been registered within the muscle tissues as increasing levels of dietary *Arthrospira (Spirulina)* result in a parallel increase of the yellowness and redness of broiler chicken meat (Toyomizu et al. 2001; Venkataraman et al. 1994). Dietary *Arthrospira (Spirulina)* levels at 1% of the total ration 1 week prior to slaughter have resulted in broiler muscle tissue pigmentation at levels best-representing consumer preferences (Dismukes et al. 2008). So, *Arthrospira (Spirulina)* has been shown to be an effective means of altering chicken product quality to meet consumer preferences. Moreover, this microalga has high antioxidant and PUFA content that enriches the nutritional value of eggs at the expense of cholesterol content as observed by Sujatha and Narahari (2011). Mariey et al. (2014) observed increased viability, improved overall health, and reduced plasma concentrations of cholesterol, triglycerides, and fatty acids in chickens fed with supplemented *Arthrospira (Spirulina)*. Moreover, chickens had an improved immune system

as demonstrated by a significant increase in white blood cell count and enhanced macrophage phagocytic activity. A generally improved health status in chickens receiving dietary *Arthrospira (Spirulina)* was also observed and was attributable to enhanced disease resistance and increased functionality of macrophage (Venkataraman et al. 1994).

Few data are available on the effect of feeding *Arthrospira (Spirulina)* in rabbits. Data from Peiretti and Meineri (2008) showed that dietary supplementation with *Arthrospira (Spirulina)* did not influence the growth rate of animals but increased the feed intake and, at dietary levels of 1% of total dry matter, improved crude protein digestibility in rabbits fed both low- and high-fat diets compared to those fed the control treatment. Similarly, *Arthrospira (Spirulina)* inclusion in growing rabbit diets did not exhibit substantial effects on growth performance, apparent digestibility, or on health status according to Gerencsér et al. (2014). Nevertheless, the 5% *A. platensis* supplementation in rabbit diets positively affected the composition of the caecal microbiota resulting in a significantly higher amount of Bacteroides and a lower amount of Clostridia (Bagóné Vántus et al. 2018). In addition, the same inclusion level was able to fortify by twofold the rabbit meat with vitamin B<sub>12</sub> (Dalle Zotte, Cullere, Sartori, Dal Basco, et al. 2014), to increase meat redness and yellowness (Dal Bosco et al. 2014), as well as  $\gamma$ -linolenic acid content (Dalle Zotte, Cullere, Sartori, Szendrő, et al. 2014).

## SWOT analysis

### Strength

Cultivating feed protein in close intensive systems instead of using croplands might help to mitigate some of the environmental and climatic impacts of feed production. The nutritional value of a microalgal-based diet is related to its ability to supply essential macro- and micro-nutrients to the target animal consumer. Many different SCP already plays a crucial role in animal feeding even if only a handful of species have been selected primarily for ease of cultivation with relatively low annual production: *Arthrospira* (3000 tons), *Chlorella* (2000 tons), *Dunaliella* (1200 tons), *Nostoc* (600 tons), *Aphanizomenon* (500 tons), *Haematococcus* (300 tons), *Cryptocodium* (240 tons) and *Schizochytrium* (10 tons) and estimated price is US\$8000–US\$300,000 per ton of dry biomass (Tibbetts 2018). Besides providing a source of protein, amino acids, fatty acids, vitamins and minerals, microalgae provide also other biologically active phytochemicals.

So, their role in feed formulation is far beyond the supply of nutrient (Hussein et al. 2013).

Microalgal biomasses have the advantage to grow in a wide range of habitats and do not spoil arable land and limit the water use for their cultivation while resulting in high yields (Kain and Destombe 1995). Some species exhibit several folds higher biomass productivities per unit area than plants (Grossmann et al. 2019). Their energy efficiency (food energy output, kg/energy input, kg) is five-fold higher than soy, twice that of corn, and over 100 times higher than grain-fed beef (Habib et al. 2008).

Moreover, scientific studies have proven high plasticity of the chemical characteristics according to cultivation and processing technology.

### Weaknesses

The availability of a consistent amount of microalgal biomass is still the main obstacle to their application for feed purposes. Moreover, despite the fact that some microalgal species are relevant for industrial applications, including commodity protein production (Tibbetts et al. 2017) and in some cases an International Feed Number has been assigned (e.g. IFN 5-20-658), standard nutrition references report (National Research Council 2011) does not exhibit data of their general composition, amino acid profile and nutrient digestibility, thus adding uncertainty for their use for feed formulation (Görs et al. 2010). Consequently, many of the nutritional claims still lack scientific evidence.

According to the known structural and compositional characteristics of algal biomass, it should be expected that ruminants are among the most suitable livestock for microalgae dietary inclusion as protein and energy supply, due to their unique digestive system. However, microalgae protein naturally appears to have high resistance against ruminal microbial degradation (Wild 2019) due to the presence of rigid and thick cell walls. The gas production and the total production of volatile fatty acids were reduced for different microalgae genera (*Chlorella*, *Nannochloropsis*, *Phaeodactylum* and *Arthrospira*) which was accompanied by high ruminal undegradable protein levels, indicating low ruminal fermentation. The availability of microalgae nutrients to animals can be limited not only by the presence of thick cell walls (*Chlorella* spp.), but also by the presence of extracellular polysaccharides (*Dunaliella tertiolecta*) that negatively affect the activity of digestive enzymes in monogastric animals (Skrede et al. 2011), compromising the intestinal health and consequently host immunity and nutrient

digestibility and uptake. The practical implication is that, in the absence of food-chain amplification, reliance on transformative intermediary organisms represents a nutritional barrier for direct feeding microalgae to most monogastric animals.

### Opportunities

Although up to 30% of the annual global microalgae supply is sold for animal feeds, many nutritional evaluation steps are still incomplete, or totally lacking, for the most microalgae-based aquafeed ingredients. Besides the nutrient content of the SCP biomass, the microalgal extracellular polysaccharides could be useful binding agents in forming feed pellets during manufacturing. Alginates are indigestible polysaccharides normally used in feeds as a stabiliser, thickener, or emulsifier agent, and as dietary bulk (Brownlee et al. 2005). Oligo-alginates have agglutination capacity that reduces leaching of water-soluble nutrients and optimise feed texture, thus improving digestibility, growth rate and developing high energy feeds (Rodriguez-Miranda et al. 2012). Anyway, the effect of the inclusion of this novel ingredient on the physical properties of compound feeds still need specific investigations. Co-products from the algae industry could result in an economically viable perspective for the utilisation of microalgae-based biomass as an alternative feed ingredient for animal feeds. With more interest in algae-derived omega-3 fatty acids as nutraceutical for both humans and animals, condensed algal residue solubles (CARS) produced from heterotrophic algae could represent a potential source of macronutrients. The CARS are produced by condensing the residue from algal fermentation of dextrose, after the extraction from the algal cell made without organic solvents. Including CARS at 5% of diet dry matter increased gain (+4.4%) and feed:gain (+10.1%), when compared to a corn-based finishing diet for cattle (Norman et al. 2019). Moreover, it has often been claimed that coupling bio-fuel production after lipids extraction from microalgae, and the utilisation of residual protein-rich biomasses in animal feeds, could represent an economically sustainable perspective in an integrated biorefinery system. However, when considering the use of such high-value products, it is necessary to avoid that the economic need to favour low-cost methods for fuel production may result in the contamination of protein residues, thus making them unsuitable for use in animal feed (Li et al. 2015). Microalgae have considered one of the favourable wastewater agents due to their ability to absorb nutrients and convert them to biomass (Chinnasamy et al. 2010), thus suggesting the



incorporation of treated wastewater-based microalgae biomass into animal feed. However, such an opportunity has received, until now, little attention due to the negative public perception, and to strict quality food regulations on animal feeds.

### Threats

Despite the efforts over the latest decades, the high production costs of microalgae still remain the main constraint to their utilisation in the animal feed industry. The use of protein from microalgae is strictly linked to its sales price and large-scale production (Sarker et al. 2016). Cell wall disruption methods can help in enhancing the efficiency of algal protein digestibility, but they must preserve the nutritional value of the several bioactive compounds inside the cell (Agboola et al. 2019). The selection of proper methods for cell wall disruption should consider not only the degree of success but also the realistic potential for an industrial scale-up (Günerken et al. 2015). Nevertheless, this further step adds in any case additional production costs. To further attenuate this situation, unlike terrestrial crops, microalgae cultivations must begin with dewatering the highly dilute cells (typically by centrifugation) down to dry biomass (typically by spray-drying) and usually some means of mechanical, chemical or enzymatic cell wall rupture is required. All these processes are currently highly energy-intensive and costly. There is no doubt that with innovation it will be possible to optimise the balance between the types and the size of the downstream processing and the associated costs. This will determine the 'yield reduction point' at which algal ingredients of the highest nutritional value, in a cost-effective way, usable for low-cost salmonid ration formulations will be obtained. In contrast to agricultural crop production, large-scale algae culture is still in its embryonic stage and production tonnage needs to dramatically rise to industrial levels to realise the benefits of economies of scale that will ensure reliable supply, consistent nutrient profile, high nutrient quality and cost-competitiveness need by deeper exploitation for the feed industry requirements. This lack of quality control and nutritional 'proofing' cannot be tolerated in quality assurance in 'from farm to fork' systems.

### Future perspectives

While it is of key importance to reduce competition with human food resources, it is also desperately needed to minimise environmental impacts and social

inequities for sustainable production of animal feeds. It already seems that microalgae will play an important role in the effort to move the animal feed industry towards a more sustainable future based on 'lower trophic' ingredients (Guedes et al. 2015). Although a limited number of studies have taken into account the beneficial effects of microalgae on health applications, as such both microalgae or as extracts, they will certainly have further applications for their bioactive compounds in animal nutrition (de Jesus Raposo et al. 2013). Considering the large discrepancy in the global supply and purchase cost of microalgal biomass versus consolidated commodity feedstuffs, improving the technologies for heterotrophic mass culture production, affordable closed photobioreactors, is mandatory. Responsible use of water resources encourages the possibility of using microalga-based water re-use systems where the 'effluent' represents a valuable resource and turns effluent 'waste' into a profitable item – while taking advantage of the SCP enhanced biomass. In the absence of high-value compounds, algal biorefineries should take a holistic approach that valorises the whole algal crop as an attractive path towards a viable microalgae-based industry, and the feed sectors are promising areas to focus on. There is tremendous potential for microalgae cultivation to be co-located with industrial point-source emitters of waste 'outputs' (e.g. CO<sub>2</sub>, nutrients, heat) which are essential 'inputs' for rapid microalgae growth and accumulation of nutrient-rich biomass. Microalgae-based ingredients could have competitive market advantages over terrestrial crops in terms of input costs, lower aerial footprint, the potential for wastewater remediation and carbon credits from CO<sub>2</sub> conversion (Apandi 2019). By cultivating microalgae in an integrated multi-system approach, it is possible to obtain, concurrently, the purification of the wastewater from other uses, while the wastewater provides free nutrients to produce the microalgal feed, as reported for *Tetraselmis suecica* (Michels et al. 2014). Microalgae have mainly been used in many biotechnological applications, where each species or strain express the required properties. The future challenge is to isolate, develop, characterise, and optimise microalgae species, or strains, that can express the specific properties relevant for animal feedstuff. Some examples are the high productivity of extractable lipids, easiness in harvesting, the use of photosynthesis by upgrading sugars, biogas or syngas from agricultural origin to produce a high-value protein that can result in higher environmental benefits.

## Conclusions

The increase in protein consumption due to the rising demand in animal products has pushed research to screen for new and environmentally friendly alternative protein sources for aquaculture and livestock. Cereals and protein-rich plants such as pulses can represent interesting and valuable options that most of the times have any legal limitations. Furthermore, these materials are often intimately linked with the territory that can be useful in renewing the perception as well as the animal production systems that use them.

The use of microalgae proteins is not a new topic but issues, such as the economically sustainable production process, are still to be solved. Despite that, these marine sources have a big potential not only in terms of biomass that potentially can be produced but also in terms of nutrients (micro/essential nutrients especially) that they can deliver. Thus, it can be concluded the plant kingdom can offer interesting opportunities requiring proper insights and evaluations to help satisfy the protein hunger of the livestock and aquaculture sectors and humanity in the future, now near.

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