A viewpoint on the use of microalgae as an alternative feedstuff in the context of 1 2 pig and poultry feeding - a special emphasis on tropical regions 3 4 Andreia A. M. Chaves, Cátia F. Martins, Daniela F.P. Carvalho, David M. Ribeiro, Madalena Lordelo, João P.B. Freire and André M. de Almeida 5 6 7 LEAF - LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da 8 Ajuda, 1349-017 Lisboa, Portugal. 9 Author for Correspondence: AM Almeida aalmeida@isa.ulisboa.pt 10 11 Abstract 12 With the current increase in meat and animal products consumption, there is a need to 13 make production systems more sustainable. The use of microalgae in monogastric feeds, replacing widely used conventional feedstuffs such corn and soybean, can be a solution 14 15 to overcome this problem. Several studies have shown promising results in the use of 16 microalgae in feeding of both pigs and poultry. However, there are several important constraints associated to the production of microalgae. Such constraints are particularly 17 limiting in the context of tropical regions. Research and scientific development on 18 microalgae production systems are thus essential so that may be widely used in 19 monogastric feeding. Herein, we conduct an overview of the major findings in the use of 20 microalgae in the context of monogastric feeding and analyse the major constraints 21 22 associated to its production and use, particularly in the specific context of tropical regions. Keywords: Microalgae, alternative ingredient, monogastric feed, swine, poultry, 23 24 challenges.

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29 Introduction

The continuous increase of world population, estimated to be over 10 billion people 30 by 2100 (UN, 2019) presents a huge challenge for worldwide food security (FAO, 2018). 31 Additionally, the increase in income and standards of living, particularly in developing 32 33 countries will consequently generate an increase in the consumption of animal products in many regions of the world, leading in turn to a higher demand for meat products. 34 35 Indeed, the growth of revenues in emerging economies will necessarily lead to higher purchasing power by local populations and, consequently, consumer choices will tend to 36 37 be more selective and less dependent on prices, generating an increase in consumption of animal products, notably meat, dairy products or eggs (EU, 2019). This increase in 38 39 demand for animal products creates a need for greater production, particularly poultry and pigs as these are the more affordable types of meat (FAO, 2011). Finally, it must be 40 41 said that such an increase in both population and demand for animal origin products is, 42 and will be particularly important in developing countries, located mostly in tropical 43 regions.

The production of monogastric and consequently of their feed, requires obtaining large 44 quantities of two ingredients: corn and soybeans, as these are the major feedstuffs 45 provided to these animals being essential sources of energy, lipids, proteins and amino 46 acids. However, these two ingredients are also very important for human nutrition. This 47 creates a problem regarding human food security and food-feed competition. Such 48 49 competition is especially important in developing countries that often do not have strong currencies reserves to ensure the purchase of these conventional feedstuffs in 50 international markets. 51

The production of conventional feedstuffs requires large areas of land. This leads in turn to habitat destruction, deforestation, and use large amounts of water, with serious consequences for the environment (Gibbs *et al.*, 2015). As such, and if the current pattern for the growth of corn and soybean production for animal and human use continues, it will, in a near future, lead to severe environmental degradation and raise sustainability issues.

58 In order to mitigate this problem, it is urgent to find novel solutions leading to higher 59 sustainability and productive efficiency in monogastric feeding and production. The use 60 of new ingredients that can be more economically and environmentally sustainable alternatives in monogastric diets is therefore the solution to overcome this problem(Tacon and Metian, 2015).

In such a context, microalgae are often suggested as interesting alternative feedstuffs,
more sustainable and interesting from the technological point of view, being able to fulfil
the high protein, amino acid and energy of pigs and poultry in a more sustainable way.

The objective of this article is to make a short and critical review on the importance of microalgae in the context of monogastric feeding, namely what are their main advantages and limitations in order to determine their importance and future relevance. This analysis finally provides a perspective related to the importance of microalgae in the context of monogastric feeding in tropical regions.

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72 Microalgae as a sustainable feedstuff with high nutritional value

Microalgae are a group of microorganisms, predominantly aquatic and microscopic, uni- or multicellular, with a wide genetic heterogeneity and with numerous physiological and biochemical varieties. This group can include almost 100,000 different species (Madeira *et al.*, 2017), of which only about 30% have been studied so far (Richmond, 2004).

These microorganisms are divided into 4 main types, depending on their pigmentation, life cycle and cell structure. They include Diatoms (*Bacillariophyceae*) which includes over 10,000 species; Green algae (*Chlorophyceae*) that are found mainly in the marine environment and make up about 17,000 species. They include also Blue-green algae (*Cyanophyceae*), which comprise about 2,000 species and are responsible for fixating oxygen in the atmosphere and, finally, Golden Algae (*Chrysophyceae*) with about 1,000 species that are predominantly found in fresh waters (Schmitz *et al.*, 2012).

Their composition can vary significantly, depending on factors such as species, 85 86 production methodology, the use of enzymes, among others, but normally microalgae have interesting levels of proteins, carbohydrates, lipids, vitamins, minerals and 87 particularly bioactive compounds, such as carotenoids (Valente et al., 2020). 88 89 Interestingly, these microorganisms have levels of protein, carbohydrates and lipids 90 comparable or even higher than those of conventional feedstuffs, with crude protein content up to 71% of dry matter (Becker, 1994). Indeed and according to Valente et al. 91 92 (2020), microalgae such as Spirulina may have average protein contents of 62-72% in dry matter content whereas for Chlorella vulgaris and Nannochloropsis oceanica protein 93

contents may be of respectively 67% and 34%. Regarding the lipid content, in Spirulina, *Chlorella vulgaris* and *Nannochloropsis oceanica*, levels may be of respectively 11, 14
and 10%. Another interesting characteristic is the mineral content that may be as high as
35% for instance in *Nannochloropsis oceanica*. Another interesting characteristic are the
PUFA (Polyunsaturated fatty acids) concentrations that can be as high as 44% of total
fatty acids in Spirulina (Valente et al., 2020).

100 Depending on how they obtain the necessary energy to survive, microalgae can be separated into two groups: autotrophic species that use sunlight and Carbon dioxide as a 101 102 source of energy and carbon, respectively, and heterotrophic species that use organic 103 carbon as an energy source (Madeira et al., 2017; Valente et al., 2020). This capacity and 104 its ubiquitous presence in ecosystems make them very important for Carbon fixation 105 reducing Carbon dioxide in the atmosphere. In fact, they are able to produce more oxygen 106 than all plants existing on the planet, representing at least 60% of Earth's Oxygen production (Chisti, 2004). Their ability to fixate Carbon present in the atmosphere 107 108 consequently lowering the greenhouse gases (GHG) balance emitted by several industries 109 is one of the interests in these species. By fixating Carbon dioxide in the atmosphere, they 110 can thus be a very useful tool for industries to reduce their ecological footprints. As such, 111 industries producing these microorganisms will simultaneously reduce the balances of GHG emissions into the atmosphere and create a product (the microalgae) that can be 112 used for instance by the food, feed or cosmetic industries, in addition to other putative 113 114 uses. It has been estimated that 30% of global microalgae production is used in feed 115 manufacture (Guill-Guerrero, 2004).

All these characteristics have led the use of microalgae in animal feed to be strongly 116 117 encouraged in the last decade. Their uses include for instance the supplementation of 118 nutrients (Valente et al., 2019; Tibaldi et al., 2015; Cardinaletti et al., 2018; Qiao et al., 119 2014 ; Kiron et al., 2016 ; Sørensen et al., 2017; Lum et al., 2013 ; Lamminen et al., 2017; Lamminen et al., 2019; Hopkins et al., 2014; Wang et al., 2017). However, in 120 121 recent years, microalgae's interest as an important source of compounds like fatty acids, pigments, vitamins and minerals has also been highlighted (Hemaiswarya et al., 2011; 122 Tibbetts, 2018; Valente et al., 2020). 123

In conclusion, microalgae, in addition to being an interesting nutritional alternative to corn and soy in monogastric feeding, may also represent an interesting solution to address sustainability concerns. Indeed, by reducing the production of ingredients such as corn and soybeans, we will contribute to lower resource consumption in agriculture. Furthermore, by using microalgae to fixate Carbon dioxide originating from human
activities, we will significantly contribute to increase the sustainability of monogastric
production systems.

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132 Microalgae in swine and poultry nutrition

Based on the above, it is of the utmost importance for the animal production sectors to improve the sustainability associated with the different production systems. The use microalgae as alternative feedstuffs is thus growing in importance. As a specific practical example, we show in figure 1, the effect of the inclusion of microalgae Tetraselmis sp. in piglet feeding at 0, 5, 10 and 15% inclusion rates. It is noteworthy to highlight the differences in pigmentation of the different feeds according to the different levels of inclusion.

As such, and based on worldwide *per capita* meat consumption patterns, their competitive costs and relatively fast production cycles, the most prominent monogastric species are undoubtedly pigs and poultry (Ritchie *et al.*, 2017). Recently, this topic has been thoroughly reviewed by us (Valente *et al.*, 2020). Readers are directed to such publication for further details.



Figure 1 – An example of the use of the microalga *Tetraselmis* sp. in a swine feeding
experiment. A – Spray-dried *Tetraselmis* sp.; B – Control diet with 0% level of inclusion
of *Tetraselmis* sp.; C – Diet with 5% level of inclusion of *Tetraselmis* sp.; D - Diet with

10% level of inclusion of *Tetraselmis* sp. and E - Diet with 15% level of inclusion of *Tetraselmis* sp.

Briefly, numerous studies on the incorporation of different dried microalgae in poultry and swine feeds have been conducted over the years (Lum *et al.*, 2013). In the case of pigs, this research is often divided according to the stage of the life cycle, clearly separating three distinct phases: post-weaning piglets, growing/ fattening pigs and breeders. In table 1, we present an overview of the main effects of the inclusion of microalgae in the different stages of pig production and in table 2, the same information for the use of microalgae in the context of poultry feeding.

- 175 Table 1 Major effects of microalgae incorporation in swine feeding (adapted from
- 176 Valente et al., 2020).

Physiological phases	Function	Main effects
Post-weaning piglets	Additive	 Improvement of feed conversion ratio ratio and weight gain. Increase of villus height in the jejunum. Increase of dry matter, N apparent total tract and ileal digestibility. Variation of effects in feces consistency. Reduce of feed intake without affecting the performance.
Growing/ Fattening pigs	Additive	 Increase of EPA, DHA and n-3/n-6 ration in muscle. Increase of dry matter digestibility. Improvement of lactobacillus intestinal population. No negative effects on animal performances. No effects in carcass characteristics and quality.
	Ingredient	Increase of EPA concentrations in muscle.No effects on performances.Increase of back fat thickness.
Breeders	Additive	 Increase of ovarian development. Increase of volume and count in sperm ejaculate. Increase of sperm mobility. Improvement of immune system of foetuses



- 185 Table 2 Major effects of microalgae incorporation in poultry, laying hens and duck
- 186 feeding (adapted from Valente et al., 2020).

Physiological phases	Function	Major effects
Broilers	Additive	 Increase of omega-3 in meat. Improvement of growth rate, performance and body weight. Increase of lactobacillus intestinal population. Improvement of immune characteristics. Improvement of fatty acids in meat. Increase of digestible methionine value. Changes in muscle color (more pigmentation).
Laying hens	Additive	 DHA enriched eggs. Change of color of yolk color (darker, orange-red, intense). Decrease of cholesterol levels in yolk. Layer performance and egg quality not affected. Enrichment of fatty acids (n-3) in eggs.
Ducks	Additive	 Improvement in body weight. No differences in performances. DHA increase in breast meat.

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In weaned piglets, the main concern is to guarantee intestinal health during post-189 190 weaning stress, by briefly avoiding diarrhoea or any factors that may delay the development and weight gain of such young animals. This phase is one of the most critical 191 192 due to the stress and drastic environmental and nutritional changes associated with weaning. It is also the stage when the animal will be most susceptible to stress factors. 193 194 Several studies have been conducted using microalgae, mostly with a prebiotic function 195 (alone or in combination with enzymes) in order to promote intestinal health and decrease 196 antibiotics use or use them as a feedstuff (Heo et al., 2013; Martins, et al., 2020).

197 In growing and fattening pigs, studies on the use of microalgae have a different role 198 and researchers frequently target them as an alternative source of proteins and 199 carbohydrates (Valente *et al.*, 2020). Indeed and as these are older animals, their 200 susceptibility to infections is lower, for that reason the use of microalgae is more frequent than in piglets. Several authors have developed works using microalgae as an adequate 201 202 protein source (Hintz et al., 1967; Neuman et al., 2018) describing its use as generally 203 beneficial. Other effects have been reported: increased linoleic acids in subcutaneous fat 204 (Altman et al., 2019), increased thickness of body fat, without negatively influencing the 205 animal's performance (Hintz et al., 1967), improved nutritional properties of meat, 206 namely the fatty acid profile in pork (Marriott et al., 2002; Moran et al., 2018; Sardi et al., 2006; Tonnac et al., 2018). In addition, the use as a prebiotic has been studied at this 207 208 stage. Yan et al. (2012) for instance reported an improvement in the microbiological 209 profile of animal faeces with microalgae incorporation.

210 In the case of breeding boars and sows, the use of microalgae is directed mostly to the 211 latter aspect. However, few studies have been done yet and the results have to be further 212 explored in order to have more sound data (Valente et al., 2020). However, the incorporation of microalgae in the feed provided to boars showed significant 213 214 improvements both in the mobility and quantity of sperm, as well as in the increase in the 215 ejaculate volume (Andriola et al., 2018; Murphy et al., 2017). The way the reproductive 216 cycle of gilts is affected by the incorporation of microalgae is still largely unexplored. 217 However, some studies show that the incorporation of microalgae in feeds during pregnancy and lactation leads to improvements in the immune system of foetuses 218 (Valente et al., 2020). 219

220 Concerning poultry, the incorporation of several species of microalgae in feeds has 221 been widely studied. In general, results indicate that when microalgae are incorporated at 222 levels below 15% have the potential to be used in several species of birds for meat 223 production, overall improving meat quality, without decreasing animal productive 224 performance (Valente *et al.*, 2020).

Studies carried out in poultry have shown that relatively small incorporations of
microalgae (less than 15%) generally lead to positive effects in several productive aspects.
They include using them as a replacer for conventional protein sources. In such cases,
animal performance parameters, digestibility, gastrointestinal development and
growthtract or traits of meat quality has generally improved (Evans *et al.*, 2015; Kang *et al.*, 2013; Park *et al.*, 2018; Shanmugapriya *et al.*, 2015; Venkataraman *et al.*, 1994; Choi *et al.*, 2017; Yan and Kim, 2013; Mirzaie *et al.*, 2018).

Studies involving the incorporation of microalgae in diets supplied to laying hens havealso shown promising results, mainly in egg quality. The fatty acid profile of both yolk

and egg white showed improvements without harming animals and even improving zootechnical indexes (Manor *et al.*, 2019; Ao *et al.*, 2015; Kralik *et al.*, 2020; Ginzberg *et al.*, 2000). There was a decrease in the yolk cholesterol contents (Park *et al.*, 2015) and an intensification of its colour (Anderson *et al.*, 1991). Improvements in eggshell hardness was also reported, decreasing the number of broken eggs in laying hens (Saeid *et al.*, 2016).

Other species of poultry, such as Japanese quails or ducks, have also been used in studies involving the improvement of animal performance, changes in skin colour, improvement of meat quality and fatty acid profiles of meat (Valente *et al.*, 2020).

Considering what was stated above, the studies carried out so far on the use of microalgae as a feedstuff for swine and poultry, are a clear indication of their interest and usefulness. However, with the increase in microalgae use, a very important drawback persists. Indeed, the production of microalgae still has a very high cost, both in the creation of structures and in the technological means necessary for their production, drying, conservation, storage and transport. All of these factors will condition the interest of microalgae as a feedstuff of commercial use.

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251 Major limitations about the use of microalgae in pig and poultry nutrition

Even with the high potential of microalgae use as in carbon fixation, production on a commercial scale is still incipient (FAO, 2009). This is due several drawbacks associated with the production systems of these organisms.

255 The first limitation is the high investment required in the installation of structures for the production of microalgae. The costs of implementing infrastructures such as closed 256 257 photobioreactors are extremely high, representing an increased initial investment that not 258 all industries and countries are currently able to support. Despite this, these reactors are 259 undoubtedly those with the highest production efficiency and with a lower energy and water use (Amin et al., 2009; Nogueira, 2010). In order to reduce these costs, more 260 261 economical structures such as open reservoirs can be used. They lead however to a 262 decrease in microalgae production due mainly to the variable environmental conditions 263 to which they are subjected to. These several contaminations and the loss of water due to 264 evaporation (Amin et al., 2009; Schenk et al., 2008; Demirbas, 2011).

The second limitation are the inputs necessary for the cultivation of these microorganisms and the high price associated to them. Indeed, the photosynthetic growth of microalgae requires sunlight, carbon dioxide, water and essential elements. These
essential elements include mainly nitrogen, phosphorus, potassium, iron, magnesium and,
in some cases, silicon. Additionally, the water used as a culture medium (usually
seawater) must be enriched by adding, for example, nitrates or fertilizers with phosphate
and other micronutrients. In addition, the optimal growth temperatures should vary
between 20 and 30°C (Molina-grima *et al.*, 1999; Iira *et al.*, 2012) leads to the need for
energy and technology to heat cultures.

Thirdly, the biomass produced has a reduced concentration (1 to 5 g / L). Indeed, and although microalgae have a fast growth rate, a good adaptive capacity to extreme environmental factors namely poor quality water, the fact of having a reduced biomass concentration leads to high prices associated to the harvest. As such, the production rate is often lower than desired (Benemann, 1997; Li, 2008).

Finally, the production of microalgae involves large energy costs in drying and conditioning the biomass produced. However, these are dependent on the efficiency of the harvest, an aspect that can easily be improved (Matos *et al.*, 2013).

All of these factors mean that the large-scale commercial production of microalgae is 282 283 still a long way from being available for animal production needs. Interestingly, the 284 energy and transport sectors may be two of the biggest promoters of microalgae increased 285 production. Indeed, the increasing need to find alternatives to fossil fuels has led to major 286 interests in the production of biodiesel using microalgae for that purpose (Barata, 2016). 287 This may provide a stimulus towards increased production and, consequently, availability 288 for other uses, namely in animal feed. Nevertheless, it is a double-edged sword, as it will also lead to competition between the two sectors. 289

As such, it is essential to carry out research envisaging to lowering production costs, increasing productivity, recycling nutrients and water, using by-products and the reusing effluents treated as carbon sources for these crops (FAO, 2010). Furthermore and in the developing countries perspective, efficient and solid translation of research results and commercial implementation of such solutions will be a dire necessity for the use of microalgae in animal feeding.

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297 Conclusions and future prospects

As mentioned above, the difficulties inherent to the use of microalgae in the context of monogastric feeding are still very significant. To some extent, such technical difficulties and their commercial price that is still very high may hamper their regular use 301 in the context of animal feeding, particularly in pigs given the fact that higher amounts 302 are needed to feed this animals by comparison to poultry. Furthermore, these difficulties 303 are a particularly important challenge in developing countries, mainly located in tropical 304 regions, due to technical and investment difficulties. Indeed, the technology required for 305 the production of microalgae on a large scale is relatively complex from a technical point 306 of view and expensive regarding the costs of construction, maintenance and operation of 307 such production units. In addition, their operating energy costs and especially the high microalgae drying and transport costs also raise questions on their viability, in both 308 309 developed and developing countries. However, in the latter and given some weaknesses 310 in terms of infrastructure and its functioning, the problem can be particularly serious.

311 To conclude, it is essential to invest in research so that novel, more economical and energetically sustainable, techniques can be used in the manufacture, drying and 312 313 transportation of microalgae ultimately targeting their use in monogastric feeding. 314 Concerning tropical developing countries, it is clear that these solutions will have to be 315 particularly adapted to several local constraints such as the use of simple reactors, 316 furthermore easy to maintain and operate that should be produced at low costs. Additionally, the use of equally technically simple, produced at low cost and using 317 renewable energy sources and not fossil fuels dryers is also a top priority. Ultimately, and 318 from the point of view of animal feed research, it is also important that the use of 319 320 microalgae moves from reduced inclusions (which has been the bulk of the research carried out so far) to higher inclusion percentages, making microalgae a possible technical 321 322 alternative to corn and soybeans in monogastric diet formulations.

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324 **Declarations**

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DMR, DFPC and ML conducted the literature search, data analysis and interpretation.
AAMC, JPBF and AMA wrote the manuscript. All authors agreed on the final version of
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