Trends in Food Science & Technology 54 (2016) 74–84

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: http://www.journals.elsevier.com/trends-in-food-science-and-technology

Review

Seaweed as a protein source for mono-gastric livestock

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ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form
17 May 2016
Accepted 20 May 2016
Available online 2 June 2016

ABSTRACT

Background: Seaweeds are often cited as alternative protein sources for livestock due to their global distribution, nutritional profile and independence from terrestrial agricultural resources.
Scope and approach: Here, we critically appraise the literature and quantitatively assess seaweeds as a protein source in livestock feeds by assembling a database of amino acid data for 121 seaweed species and comparing the quality and concentration of protein to 'traditional' protein sources (soybean meal and fishmeal) and then benchmarking the seaweeds against the amino acid requirements of mono-gastric livestock (chicken, swine and fish).
Key findings and conclusions: The quality of protein (% of essential amino acids in total amino acids) of many seaweeds is similar to, if not better than, traditional protein sources. However, seaweeds without exception have substantially lower concentrations of total essential amino acids, methionine and lysine (on a whole biomass basis, % dw) than traditional protein sources. Correspondingly, seaweeds contain an insufficient concentration of protein, and specifically insufficient essential amino acids, to meet the requirements of most mono-gastric livestock in the whole seaweed form. Consequently, the concentration or extraction of protein from seaweeds will be the most important goal in their development as an alternative source of protein for mono-gastric livestock.

1. Introduction

Protein for mono-gastric livestock, non-ruminants including chickens, swine and fish, is mostly provided through compound diets with soybean meal and fishmeal as the major sources (Boland et al., 2013). However, the food security of these protein ingredients is a growing concern due to an increasing world population (Godfray et al., 2010), increasing demand for ingredients to supply livestock protein (von Braun, 2007), limited agricultural resources of arable land and fresh water (Pretty, 2008), declining wild fish stocks (Tacon & Metian, 2008) and competition with biofuels (Nigam & Singh, 2011). Consequently, there is a critical role for alternative crops in securing the future supply of protein (Boland et al., 2013).

Seaweeds (marine macroalgae) are often proposed as an alternative protein crop for use in compound diets for animals as they have high crop productivities per unit area (Bolton, Robertson-Andersson, Shuuluka, & Kandjengo, 2009; Mata, Magnusson, Paul, & de Nys, 2016; Mata, Schuenhoff, & Santos, 2010; Nielsen et al., 2012) and do not require arable land or fresh water. These rationale have, at least in part, contributed to the recent spike in the number of feeding trials that have included whole seaweeds in compound diets at high inclusion rates (20–30%), particularly for aquaculture livestock (Felix & Brindo, 2013; Marinho et al., 2013; Pereira, Valente, Sousa-Pinto, & Rema, 2012; Stadtlander, Khalil, Focken, & Becker, 2013; Shapawi, Safiin, & Senoo, 2015). However, there has been no systematic analysis of which seaweeds should be targeted based on the quality (% amino acids as a proportion of protein) and concentration (% protein on a whole biomass basis) of protein, which to date are mostly reported independently (Fleurence, 1999; Fleurence et al., 2012). The analysis of the quality and concentration of protein is critical in determining the nutritional value of whole seaweed biomass if it is to be used in its whole form as an ingredient. This analysis is important as it provides the concentration of essential amino acids on a whole biomass basis and is the foundation for the nutritional assessment of a protein source. Furthermore, any new protein source needs to be assessed on the provision of the most-limiting essential amino acid on a whole weight basis relative to the requirements of the target

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http://dx.doi.org/10.1016/j.tifs.2016.05.014

livestock, preferably where ileal digestibility (amino acid digestibility at the end of the intestine, corrected for basal endogenous amino acid losses) is accounted for (Leser, 2013; Stein, Seve, Fuller, Moughan, & De Lange, 2007). Finally, the low digestible energy content of seaweeds needs to be considered as an indirect factor as to whether the whole seaweed biomass can be used to replace other ingredients as a protein source in compound diets for mono-gastric livestock. The cumulative effect is that seaweeds cannot be assessed solely on the quality or concentration of protein, but rather on the concentration of the limiting essential amino acids for each type of livestock.

Therefore, the aim of this review is to quantitatively assess the potential of seaweeds as a protein source for mono-gastric livestock. To do this, all available literature on the amino acids in seaweeds was systematically analysed to create, and make publically available (Angell, de Nys, & Paul, 2015a), the first comprehensive data set on the quality and concentration of amino acids in seaweeds. This data set contains 265 seaweed samples representing 121 species from 45 peer-reviewed articles (see Supplementary Material for methods). We compare the quality and concentration of protein in these seaweeds to the two traditional protein sources – soybean meal and fishmeal. Subsequently, we compare each seaweed, and both traditional protein sources, to the requirements of mono-gastric livestock to determine the concentration of the limiting essential amino acid as a diet for chickens, swine and fish (salmon and tilapia). This quantitative data, in conjunction with a review of published feeding trials using seaweed, is used to assess positive and negative aspects of incorporating seaweeds in a whole form in the compound diets of mono-gastric livestock. Finally, we propose the concentration and isolation of protein to make seaweeds more accessible as an ingredient in compound diets and define a path for future research.

2. The quality of protein in seaweeds

Mono-gastric livestock do not have a requirement for protein *per se* but rather for the amino acids from which proteins are made. It is those essential amino acids (Table 1) that cannot be synthesised by livestock that are critical in the diet and define the quality of a protein source. Seaweeds have a relatively high quality of protein (essential amino acids as a proportion of total amino acids (TAA)) compared to fishmeal and soybean meal. Overall, seaweeds have similar or higher proportions of total essential amino acids (EAA) (mean = 45.7% TAA) compared to fishmeal (43.4% TAA) and soybean meal (46.0% TAA). More than 75% of seaweeds have higher proportions of total EAA than fishmeal and 50% are higher than soybean meal (Fig. 1A).

The proportion of the essential amino acids methionine and lysine (% TAA), the limiting amino acids in most commercial diets and regularly supplemented artificially (NRC, 1998, 2011), is comparable to traditional protein sources. Seaweeds (mean = 1.84% TAA) are generally superior to soybean meal (1.25% TAA) in the proportion of methionine, but generally have a lower proportion of methionine than fishmeal (2.8% TAA) (Fig. 1B). More than 75% of seaweeds have a higher proportion of methionine than soybean meal. On the other hand, most seaweeds have a lower proportion of lysine than soybean meal (6.66% TAA) and fishmeal (7.3% TAA), although some species have higher values than either of these protein sources (25 species with a proportion of lysine > 7.3% TAA) (Fig. 1C).

Red seaweeds typically have a higher quality of protein than brown and green seaweeds. Red seaweeds have the highest mean proportion of total EAA and lysine and the second highest mean proportion of methionine compared to brown and green seaweeds (Fig. 1A–C). However, there is substantially more variation between species of seaweeds within the taxonomic groups (red, green or brown seaweed) than between the taxonomic groups. Therefore, broad taxonomic groupings provide little certainty in selecting species with a high quality of protein.

The data for the quality of protein in this review confirms the conclusions reached by many authors that seaweeds generally have a comparable, if not superior, quality of protein to traditional protein sources.

2.1. The concentration of essential amino acids in seaweeds

The assessment of a protein source to provide nutritional value relies on an analysis of the quality of protein and the concentration of protein if the seaweed is to be used in a whole form as a feed for any particular livestock. The concentration of essential amino acids as a % of the whole biomass on a dry weight basis (dw) takes into account the quality of protein (essential amino acids as a

Table 1
The concentration of protein and essential amino acids (% dw) of traditional protein sources, other feed ingredients and seaweeds. The concentration of essential amino acids, specifically those that are often limiting in the diets of mono-gastric livestock (i.e. lysine and methionine), determine how much protein can be utilised by mono-gastric livestock.

% dw	Fish meal[a]	Soybean meal[b]	Corn grain meal[c]	Seaweed	
				Mean	Range
Protein	68.70	48.00	10.20	11.60[d]	2.98–26.76[d]
Arginine	3.71	3.60	0.40	0.73	0.02–2.99
Histidine	1.55	1.30	0.25	0.24	0.00–1.02
Isoleucine	3.35	2.60	0.29	0.54	0.01–1.78
Leucine	4.85	3.80	1.00	0.92	0.03–3.18
Lysine	6.21	2.24	0.26	0.69	0.02–2.12
Methionine	2.08	0.70	0.18	0.20	<0.01–0.69
Methionine + cystine	3.19	1.41	0.37		
Phenylalanine	2.67	2.70	0.42	0.61	0.02–1.83
Phenylalanine + tyrosine	4.80	3.95	–		
Threonine	2.66	2.00	0.30	0.61	0.02–2.11
Tryptophan	0.72	0.70	0.07	0.10	0.00–0.27
Valine	3.39	2.70	0.42	0.68	0.02–2.45
Total EAA (% dw)	31.19	22.34	3.59	5.49	0.15–16.35

[a] NRC (2011) – Mean of anchovy and herring fishmeal, protein value is crude protein.
[b] NRC (2011) – Solvent extracted without hulls, protein value is crude protein.
[c] NRC (2011) – Protein value is crude protein.
[d] Angell et al. (2016) – mean and range based on the 5th/95th percentile range of protein determined by total amino acid analysis (n = 299 red, green and brown seaweeds).

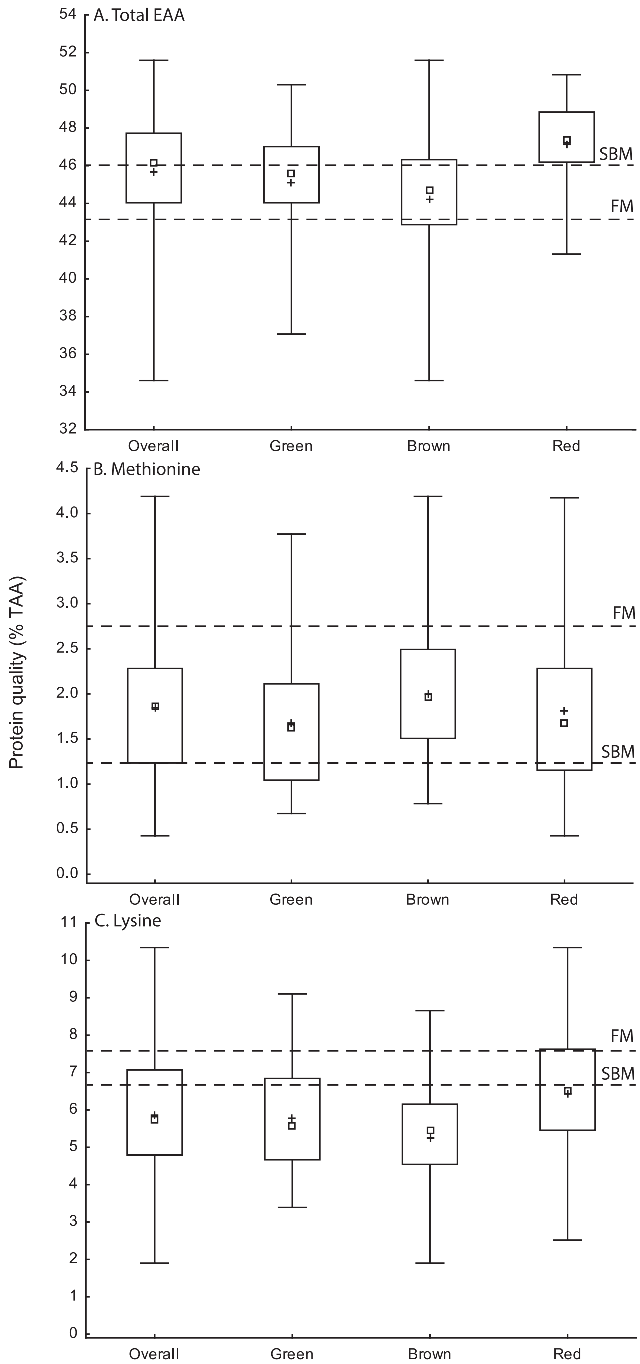

Fig. 1. The proportion of (A) total essential amino acids, (B) lysine and (C) methionine of TAA for seaweeds analysed in this review compared to concentrations in major feed ingredients. Squares represent medians, crosses represent means, boxes represent 25th percentiles and whiskers represent minimum/maximum. FM = fishmeal and SBM = soybean meal.

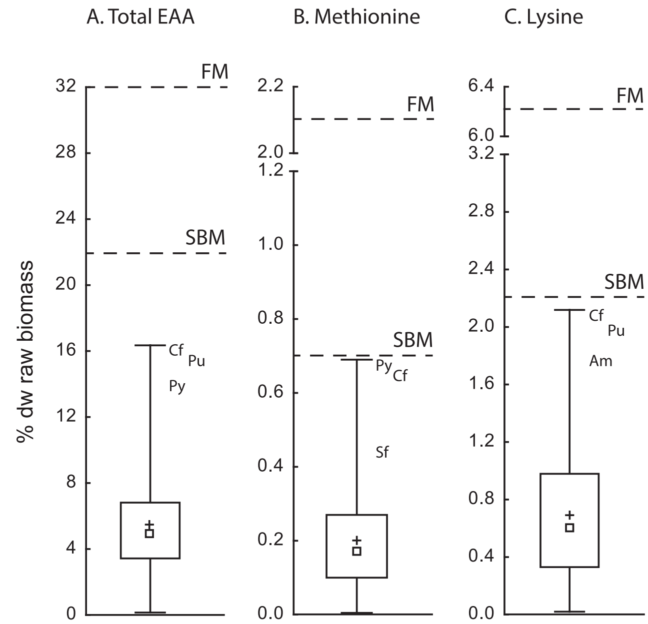

Fig. 2. The dry weight concentration (% dw) of (A) total essential amino acids, (B) methionine and (C) lysine of seaweeds analysed in this review compared to concentrations in major feed ingredients (see Table 1). These amino acids are most often artificially supplemented in plant based diets for domesticated livestock. Squares represent medians, crosses represent means, boxes represent 25th percentiles and whiskers represent minimum/maximum. Dashed lines represent concentrations for soybean meal (SBM) and fishmeal (FM). Seaweeds with the highest concentrations indicated by Cf = *Capsosiphon fulvescens*, Pu = *Porphyra umbilicalis*, Py = *Pyropia yezoensis*, Sf = *Solieria flliformis* and Am = *Amansia multifida*.

proportion of protein or TAA) and the concentration of protein in the biomass.

The concentration of total essential amino acids (total EAA % dw) in seaweeds (5.49%) is substantially lower than in soybean meal (22.34%) and fishmeal (31.19%) (Table 1, Fig. 2A). However, there is considerable variation in the concentration of total EAA among seaweeds, with the maximum value reported (16.35% dw) three-times that of the mean (5.49% dw). Similarly, the concentration of the essential amino acids methionine and lysine (% dw) are substantially lower in seaweeds than in soybean meal and fishmeal. More than 80% of seaweeds have less than half the concentration of methionine and lysine than that of soybean meal and fishmeal (Table 1, Fig. 2B and C). These differences are consistent for all individual essential amino acids for soybean meal and fishmeal – with the exception of threonine (Table 1).

The low concentration of EAAs in seaweeds (% dw) highlights the problem of generalising that whole seaweed can be used effectively as a protein source. We acknowledge this contradicts the accepted paradigm that many seaweeds have a high concentration of protein with high proportions of essential amino acids (Fleurence, 1999; Fleurence et al., 2012; Garcia-Vaquero & Hayes, 2015). However, protein concentration calculated for seaweeds are often overestimated by the use of the animal-based 6.25 N-to-protein conversion factor, and more accurately should be determined using a N-to-protein factor of 5.0 (Angell, Mata, de Nys, & Paul, 2016). In many instances the low concentration of protein is rarely identified when presenting amino acid profiles as a ratio to protein or TAA (Table 1). Furthermore, the true value of seaweeds as a protein source needs to be assessed on a per livestock (domesticated species) basis based on the provision of the limiting essential amino acid. This is because the essential amino acid requirements vary substantially between mono-gastric livestock and between different stages of their production (Table S1).

3. Limiting essential amino acids

The concentration of each essential amino acid in a protein source (on a % dw basis) relative to that of the livestock requirements (% dw basis) can be used to calculate the maximum potential (before digestibility is accounted for) of the protein source to provide the essential amino acids for that particular livestock.

The amino acid score, defined as the smallest ratio of any of the 10 essential amino acids, sets the limiting essential amino acid and this ratio determines how much protein or TAA can be utilised by the livestock (amino acid score – Eq. (1)). An amino acid score was calculated for each seaweed species examined in this review for each livestock (chicken, swine, salmon and tilapia) based on using seaweed as the sole protein source (see Supplementary Material for methods).

$$\text{Amino acid score} = \frac{\text{Concentration of 1st limiting EAA in protein source } (\%\ dw)}{\text{Livestock requirement of limiting EAA in diet } (\%\ dw)} \quad (1)$$

The five essential amino acids methionine, arginine, histidine, tryptophan and lysine are the limiting amino acids for 96% of seaweed species for chickens, swine, salmon and tilapia. Furthermore, these amino acids also account for more than 80% of the second limiting amino acid and more than 30% of the third limiting amino acid. Methionine is most frequently the limiting essential amino acid for all major mono-gastric livestock (33–49% of seaweed species), with the exception of tilapia which is most commonly limited by histidine (53% of species) (Fig. 3). This contrasts with terrestrial plant sources that are commonly limited by lysine (NRC, 1998, 2011). In contrast, lysine is rarely limiting for seaweeds, especially for chickens and tilapia where methionine, arginine, histidine and tryptophan are the main limiting amino acids for these livestock (Fig. 3). Notably threonine, which is often artificially supplemented in commercial diets with traditional protein sources (NRC, 1998, 2011), is rarely limiting for seaweeds for any livestock. Tryptophan, which was only measured in 37% of seaweeds, is the limiting amino acid for between 15% (salmon) and 38% of species (mature swine). This supports that tryptophan should be reported even if its measurement requires a different analysis.

Ideally, the amino acid scores calculated above should be adjusted for ileal digestibility, that is, the amino acid outflow at the end of the intestine, corrected for basal endogenous amino acid losses (Stein et al., 2007). This would then enable the calculation of a Digestible Indispensible Amino Acid Score – DIAAS (Leser, 2013). However, standardised ileal digestibility data for amino acids in seaweeds have not been reported so we provide a best case scenario (assuming 100% digestibly) to assess the potential of seaweeds to provide protein nutrition to mono-gastric livestock. While it is difficult to predict what these scores would be after accounting for digestibility, amino acid scores above 1.50 are presumed to be high enough to balance losses due to digestibility inefficiencies, considering that the true ileal digestibility of essential amino acids for the majority of commonly used feed ingredients for swine range from 60 to 95% (NRC, 1998).

Amino acid scores, calculated based on the assumption of 100% digestibility (Eq. (1)), for the majority of seaweeds are low for all livestock and lower than traditional protein sources (Fig. 4). This suggests that these seaweeds cannot satisfy the amino acid requirements of mono-gastric livestock even in the best case scenario for digestibility. This was most evident for fish (salmon and tilapia) where no seaweed species has an amino acid score above 0.70 (70% of essential amino acid requirements met before losses due to digestibility). Furthermore, only a few seaweeds have amino acid scores above 1.00 (100% of essential amino acid requirements met) for young chickens, young swine and mature chickens, and none have a score greater than 1.50 (150% of essential amino acid requirements met) (see Table S4). The best outcome is for mature swine where seven seaweed species have amino acid scores higher than 1.50 (Table S4). However, these scores also assume 100% inclusion in the diet and does not account for the inclusion of an energy source (e.g. corn) that usually represents approximately 75% of the diet for swine (NRC, 1998) and has a poor concentration of essential amino acids (Table 1). It is also evident that the amino acid scores of the best seaweeds (1.52–2.50) are still considerably lower than those for soybean meal (3.67) and fishmeal (6.55) (Fig. 4). This does not mean that they should be overlooked, however, considerable effort will need to be focussed on the industrial production and processing of seaweeds to make them competitive as a

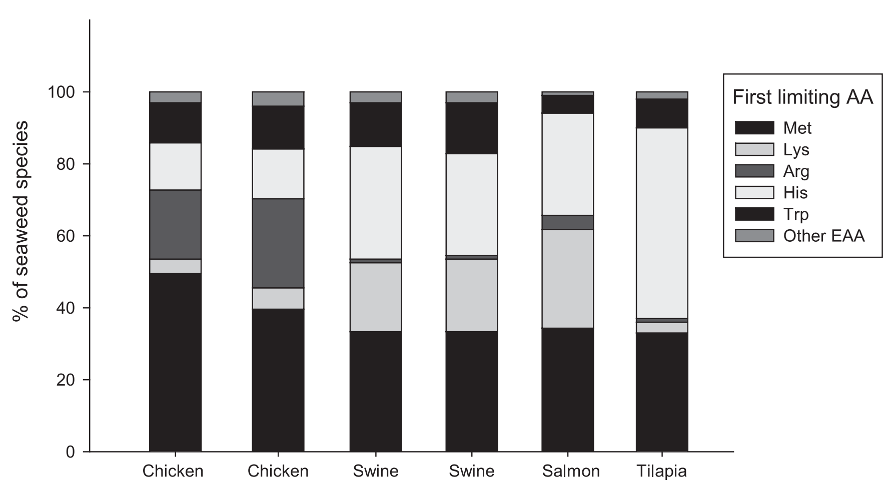

Fig. 3. Proportion of seaweed species that were limiting in each essential amino acid for mono-gastric livestock. Amino acid requirement data for chickens, swine and fish from NRC (1994), NRC (2012) and NRC (2011), respectively. Met – methionine, Lys – lysine, Arg – arginine, His – histidine, Trp – tryptophan and EAA – essential amino acid.

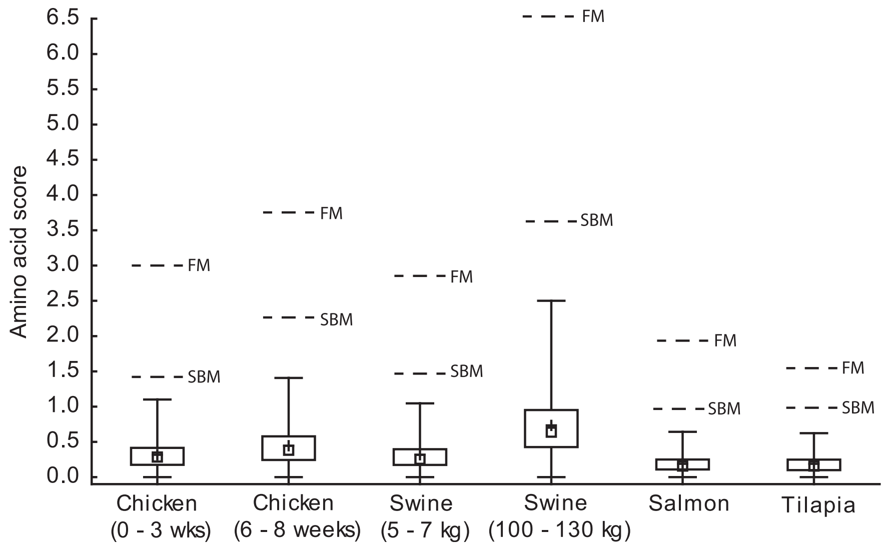

Fig. 4. Amino acid scores – defined as the ratio of the limiting AA (% dw) to the livestock requirement of the same amino acid - of seaweeds analysed in this review for major domesticated livestock. Squares represent medians, crosses represent means, boxes represent 25th percentiles and whiskers represent minimum/maximum. Dashed lines represent amino acid scores for soybean meal (SBM) and fishmeal (FM) (NRC 2011). Amino acid requirement data for chicken, swine and fish from NRC (1994), NRC (2012) and NRC (2011), respectively.

feedstock. This would be bolstered by the uncertainty in the future security and price of these traditional sources (Aiking, 2011), but the targeted development of seaweeds as a protein resource would also need to facilitate innovations in the concentration or extraction of proteins (see below).

4. The use of whole seaweeds in mono-gastric livestock diets

Most of the research on incorporating seaweeds into livestock diets as a source of protein has focused on aquatic livestock, especially commercial marine herbivores that feed naturally on seaweeds (abalone and sea urchins). Seaweeds can provide complete or partial protein nutrition for abalone (Bautista-Teruel, Millamena, & Fermin, 2001; Bilbao et al., 2012; Kemp, Britz, & Aguero, 2015; Mulvaney, Winberg, & Adams, 2013; Viera et al., 2011), sea urchins (Cook & Kelly, 2007) and shrimp (Cruz-Suarez, Tapia-Salazar, Nieto-Lopez, Guajardo-Barbosa, & Ricque-Marie, 2009; da Silva & Barbosa, 2009; Felix & Brindo, 2013). In contrast, the inclusion of seaweed in diets of commercial fish (herbivores or carnivores) at levels greater than 10% results in reduced growth and feed utilisation (Table 2), although there are some promising results for tilapia (Stadtlander et al., 2013). There is little literature on incorporating seaweeds as a protein source into the diets of poultry and swine. In contrast, there are many studies examining the functional effects of seaweed and their extracts on immune function, gut health, and meat and egg quality (Katayama et al., 2011; Kulshreshtha et al., 2014; Michalak et al., 2011; Walsh, Sweeney, O'Shea, Doyle, & O'Doherty, 2013; Walsh, Sweeney, O'Shea, Doyle, & O 'Doherty, 2013). However, these studies use low inclusion levels (<5%) that contribute little protein to the diet or, its corollary, that the seaweeds do not displace traditional protein sources in the feed. The few studies that have incorporated seaweeds into poultry livestock diets at levels higher than 5% report some positive results (no negative effect on growth or feed utilisation) up to 10% for chickens (Ventura, Castanon, & Mcnab, 1994; Zahid, Aisha, & Ali, 1995) and up to 15% for ducks (El-Deek & Brikaa, 2009a,b), but reduced performance at higher inclusion levels for chickens (Ventura et al., 1994).

The potential of substituting seaweeds as a protein source into compound diets (a diet composed of multiple ingredients) is based on the assumption that it should displace a substantial proportion of the existing protein source (usually soybean meal) while maintaining the concentration of essential amino acids and the digestible energy level. This can be modelled using a theoretical example based on changes to the overall amino acid score of the diet. As a best case example, we used mature swine (the livestock with the highest amino acid scores, i.e. the lowest amino acid requirements), and focused on the three seaweeds with the highest concentration of the amino acid (% dw) that limits the swine compound diet (*Capsosiphon fulvescens* (C. Agardh) Setchell & N. L. Gardner, *Porphyra umbilicalis* (Linnaeus) J. Agardh and *Amansia multifida* J. V. Lamouroux). A typical swine compound diet consists of an energy component (corn) and a protein component (soybean meal). These two ingredients make up approximately 97.5% of the diet, with corn typically 74.1% and soybean meal 23.4% (NRC, 1998). This combination of corn and soybean meal gives an amino acid score for mature swine of 1.17 and lysine as the limiting amino acid (note that, in practice, this score will change based on losses due to digestibility and gains due to supplementation with artificial lysine). Only three species of seaweed, of the 119 examined (Table S2), have concentrations of lysine comparable to soybean meal (2.24% dw) (*Capsosiphon fulvescens* (2.12% dw), *Porphyra umbilicalis* (2.05% dw) and *Amansia multifida* (1.85% dw)) (see Fig. 2C). Therefore, these three seaweeds were used, as best case examples, to examine what effect substituting soybean meal with whole seaweed has on the overall amino acid score of the theoretical swine compound diet (74.1% corn and 23.4% soybean meal) with substitution levels from 5 to 100% of soybean meal.

By substituting the soybean meal protein source with increasing amounts of the three seaweeds with the highest lysine concentration, there is little change to the overall amino acid score of the diet as long as lysine remains the limiting amino acid (see Fig. S1 and supplementary results). However, the amino acid score of the compound diet drops dramatically as tryptophan, the limiting amino acid of the seaweed, becomes more limiting than lysine. This demonstrates that when seaweeds with high concentrations of lysine are partially substituted for soybean meal there may be some potential to use specific seaweeds in a whole form in the compound diets of livestock with low amino acid requirements, provided tryptophan is artificially supplemented. However, the majority of

Table 2
A summary of feeding trial research which have incorporated whole seaweeds as part of the diets of mono-gastric livestock.

Livestock examined	Inclusion levels tested (% of diet)	Seaweed examined (genus)	Major findings	Reference
Fish & shrimp				
Asian seabass (*Lates calcarifer*)	5	*Kappaphycus*, *Eucheuma*, *Sargassum*	• No effect on growth performance • *Sargassum* improved feed intake • Seaweeds used to replace commercial feed binders	(Shapawi & Zamry, 2016)
Asian seabass (*Lates calcarifer*)	6, 10, 14, 18, 22 (cooked) and 6 (raw)	*Kappaphycus*	• 6% inclusion of cooked seaweed improved growth and FCR compared to control and other treatments • Reduced growth and increased FCR with increasing inclusion levels above 6% • Note: seaweed replaced tapioca starch rather than the protein sources used (fishmeal and soybean meal)	(Shapawi et al., 2015)
European seabass (*Dicentrarchus labrax*)	5, 10 and 15	*Pterocladia*, *Ulva*	• Seaweed inclusion had no significant effect on growth or PER. • 5% inclusion increased performance, nutrient composition and stress resistance compared to control • Note: seaweed primarily replaced wheat flour rather than fishmeal - the major protein source	(Wassef, El-Sayed, & Sakr, 2013)
European seabass (*Dicentrarchus labrax*)	5 and 10	*Gracilaria*, *Ulva*	• FCR improved for both inclusion levels • Note: 10% reduced growth and digestibility	(Valente et al., 2006)
Nile tilapia (*Oreochromis niloticus*)	5 and 10	*Gracilaria*	• No difference in growth, FCR and PER between control and 5%, but these were negatively affected at 10% • 5% increased innate immune response	(Araújo et al., 2015)
Nile tilapia (*Oreochromis niloticus*)	13.6 and 27.2	*Porphyra*	• No effect on growth or feed utilisation	(Stadtlander et al., 2013)
Nile tilapia (*Oreochromis niloticus*)	10, 15 and 20	*Ulva*	• Inclusion above 10% decreased growth performance, protein utilisation and protein retention	(Marinho et al., 2013)
Nile tilapia (*Oreochromis niloticus*)	30	*Gracilaria*, *Porphyra*, *Sargassum*, *Ulva*	• Diet protein digestibility decreased compared to control in all seaweed inclusion diets except for *Gracilaria*	(Pereira et al., 2012)
Rainbow trout (*Oncorhynchus mykiss*)	5 and 10	*Gracilaria*	• No effect on growth or FCR at 5%, but these were negatively affected at 10% • Increased iodine and moisture content and higher colour intensity at 5%	(Valente et al., 2015)
Rainbow trout (*Oncorhynchus mykiss*)	30	*Gracilaria*, *Porphyra*, *Sargassum*, *Ulva*	• Diet protein digestibility decreased compared to control in all seaweed inclusion diets	(Pereira et al., 2012)
Rabbitfish (*Siganus canaliculatus*)	33	*Gracilaria*	• Reduced growth and feed utilisation in seaweed inclusion diet compared to control (fishmeal protein source) • Note: some immunity parameters improved in seaweed diet	(Xu et al., 2011)
Mullet (Chelon labrosus)	16.5 and 33	*Porphyra*	• Increased inclusion levels of seaweed resulted in reduced growth and compromised FCR, PER and NPU.	(Davies, Brown, & Camilleri, 1997)
Large yellow croaker (Pseudosciaena crocea)	5, 10 and 15	*Ulva*	• Inclusion up to 15% had no effect on growth or survival • Note: seaweed replaced wheat meal not fishmeal or soybean	(Asino, Ai, & Mai, 2011)
Atlantic cod (*Gadus morhua*)	5.5 and 11	*Porphyra*	• No effect on survival, growth or hepasomatic index • Note: diets were isonitrogenous and isocaloric by incorporating additional blood meal	(Walker, Fournier, Neefus, Nardi, & Berlinsky, 2009)
Rainbow trout (*Oncorhynchus mykiss*)	10	*Ulva*	• Reduced growth, feed intake and feed utilisation	(Yildirim, Ergun, Yaman, & Turker, 2009)
Atlantic salmon (*Salmo salar*)	5, 10 and 15	*Palmaria*	• No effect on red coloration of fillets • Positive effect on yellow/orange colour of fillets	(Moroney et al., 2015)
Japanese flounder (*Paralichthys olivaceus*)	3, 6 and 9	*Eucheuma*	• 3% inclusion improved growth and feed efficiency • Reduced growth and feed efficiency at inclusion levels above 6%	(Ragaza et al., 2015)
Shrimp (*Litopenaeus vannamei*)	3.3	*Ulva*, *Macrocystis*, *Ascophyllum*	• No effect on feed intake or survival, • For Ulva only, small increase in growth, improved FCR and PER	(Cruz-Suarez et al., 2009)
Shrimp (*Litopenaeus vannamei*)	13, 26 and 39	*Hypnea*, *Crytonemia*	• Increased survival • No difference in biomass and SGR • Note: fishmeal also increased with increasing amounts of seaweed	(da Silva & Barbosa, 2009)
Freshwater prawn (*Macrobrachium rosenbergii*)	10, 20 and 30	*Kappaphycus* (whole and fermented)	• No effect on growth, digestibility and flesh quality with whole seaweed (up to 20%) and fermented seaweed (up to 30%)	(Felix & Brindo, 2013)
Terrestrial livestock				
Chicken	10, 20 and 30	*Ulva*	• Reduced growth and feed intake as seaweed inclusion level increased	(Ventura et al., 1994)
Chicken	1 and 3	*Ulva*	• No effect on feed intake, growth or feed utilisation • Positive effects of 3% diet on muscle yield and serum quality	(Abudabos et al., 2013)
Duck	1.5 and 3	*Polysiphonia*	• No significant effect of seaweed on growth parameters or carcass traits • Note: pellet hardness increased with seaweed inclusion	(A. El-Deek & M. A. Brikaa, 2009)

(*continued on next page*)

Table 2 (*continued*)

Livestock examined	Inclusion levels tested (% of diet)	Seaweed examined (genus)	Major findings	Reference
Duck	5, 10 and 15	*Polysiphonia*	• No effect on growth or FCR	(A. El-Deek & A. M. Brikaa, 2009)
Swine	0.8	Not specified	• Improved immune function response	(Katayama et al., 2011)
Swine	1 and 2	*Ascophyllum*	• Improved gut health at 1%	(Dierick et al., 2009)

FCR = feed conversion ratio, PER = protein efficiency ratio, NPU = net protein utilisation.

seaweeds contain substantially less lysine than soybean meal (Fig. 2C) and, if added to a compound diet already limiting in lysine, will substantially decrease the amino acid and digestible indispensable amino acid scores of the diet. Moreover, as seaweeds contain large concentrations of fibre and ash (Al-Harthi & El-Deek, 2012; Marrion et al., 2005; Ventura et al., 1994), the digestible energy content of the compound diet will also decrease as soybean is substituted with seaweed. This suggests that whole seaweeds are too diluted by fibre and ash to maintain or improve the amino acid content of traditional compound diets without negatively affecting their energy content for even one of the most promising livestock (mature swine) when 100% digestibility is assumed.

We have established that there are clear limitations in the concentration of essential amino acids and fibre and ash of seaweed when considering whole seaweeds as an alternative to traditional protein sources of soybean meal and fishmeal feed ingredients. This dictates that the pathway forward is to concentrate the protein either by the removal of non-protein components or the extraction of protein. If this can be done then the concentration of essential amino acids (low in seaweeds) can be separated from the quality of protein (high in seaweeds). This provides a renewed focus on developing processing methods to concentrate protein in seaweeds rather than a focus on whole seaweed biomass, which has been the case in recent years (only 1 out of 50 published articles with a reference to a protein concentration of a seaweed in a nutritional context from 2012 to 2014 examined protein extracts) (Angell, de Nys, et al., 2015a).

5. Developing an alternative protein source from seaweeds

In the previous sections we established that the key limitation for the use of seaweed protein is the concentration of essential amino acids on a whole basis not the quality of the total amino acids or protein. Effectively, the large proportion of non-amino acid material (indigestible carbohydrates/fibre and ash) dilutes the high quality protein of seaweed. The processing methods for soybeans provide a model for the production of concentrated protein products for mono-gastric livestock. After extraction of lipids, residual soybean biomass (soybean meal) is further processed to concentrate protein by the removal of non-protein components (soybean protein concentrate), or by the direct extraction of proteins (soybean protein isolate) (Berk, 1992). For most seaweeds, the aim of the processes would be to concentrate protein by a factor of 75–200% to provide a comparable protein source to soybean meal.

5.1. Removal of non-protein components to concentrate protein

The simplest form of processing to remove non-protein material from seaweeds and concentrate protein is rinsing biomass with freshwater to reduce the content of ash. Ash contents are comprised of external and internal salts and usually constitute between 20 and 50% of the dry weight (Angell, Mata, de Nys, & Paul, 2015b; McDermid & Stuercke, 2003; McDermid, Stuercke, & Balazs, 2007). Therefore, processing with freshwater has the potential to increase the concentration of protein in seaweeds by an equivalent amount. This may only be suitable for those seaweeds that have a high concentration of essential amino acids as the polysaccharides, which can represent up to 76% of the dry weight (Kraan, 2012), are typically not affected by simple rinsing. However, few studies have quantified the effect of rinsing on the concentration of ash and protein. Notably, rinsing increased the concentration of protein in the siphonous green seaweed *Derbesia tenuissima* by 34%, and the green blade seaweed *Ulva ohnoi* by 15% (Neveux et al., 2014). Similarly, optimised rinsing further increased the concentration of protein from 23.4 to 27.4% (17% increase) for *Ulva ohnoi* and 15.2%–19.5% for *Ulva sapora* (28% increase) (Magnusson, Carl, Mata, de Nys, & Paul, 2016).

The extraction of polysaccharides has the potential to further increase the amino acid concentration in the seaweed biomass. Most of these polysaccharides are structural cell wall material (e.g. cellulose in green seaweed), however, unlike terrestrial plants, structural and storage polysaccharides in seaweeds are predominantly species-specific. For example, green seaweeds contain cellulose, sulphated galactans (ulvans), sulphated polysaccharides and xylans, brown seaweeds contain alginic acid, fucoidan, laminarin and sargassan, and red seaweeds contain agars, carrageenans, xylans, floridean starch, sulfated galactan and porphyran (Chiovitti et al., 1997; Kraan, 2012; Percival, 1979; Ray & Lahaye, 1995). This diversity of polysaccharides means that the extraction yield and methodology is varied and often species-specific. Extractible polysaccharide content in seaweeds range from 6.5 to 38% dw (Barros et al., 2013; Kraan, 2012; Maciel et al., 2008) and have been extracted using water-soluble extraction at room temperature (Alves, Sousa, & Reis, 2013; Kolender & Matulewicz, 2002; Maciel et al., 2008) or high temperatures (Barros et al., 2013; Yamamoto, 1980), enzymatic digestion (BobinDubigeon, Lahaye, Guillon, Barry, & Gallant, 1997; Melo, Feitosa, Freitas, & de Paula, 2002), and acidic- (fucans) and alkaline-soluble extractions (Ray, 2006). However, the protein fraction has rarely been considered and it will now be important to quantify the effects the solvents have on the extraction of proteins. For example, the by-product of the agar extraction process from *Gracilaria* potentially represents an underutilised protein resource, however, extraction procedures involve an alkaline extraction using sodium hydroxide (Armisen, 1995) that is critical in solubilising a large proportion of total soluble protein during protein extraction processes for seaweeds (Fleurence, LeCoeur, Mabeau, Maurice, & Landrein, 1995; Kandasamy, Karuppiah, & Rao, 2012; Kumar, Ganesan, Selvaraj, & Rao, 2014; Wong & Cheung, 2001a, 2001b). Although it has not yet been a commercial focus for seaweeds, it is promising that the extraction of soluble polysaccharides with minimal protein losses is routinely done for soybeans, providing a model for seaweeds (Berk, 1992).

5.2. Direct extraction of protein

The extraction of total protein from seaweeds is impeded by cell wall mucilage (neutral polysaccharides) and phenolic compounds

Table 3
Crude protein, total essential amino acid (EAA), methionine (Met) and lysine (Lys) concentration in whole seaweed and protein extracts (% dw) for different seaweeds as well as for high protein sources.

Seaweeds	Yield[a]	Crude protein		Total EAA concentration		Met concentration		Lys concentration	
		Whole	PE	Whole[b]	PE	Whole[b]	PE	Whole[b]	PE
Red									
Hypnea charoides[c]	46.3	18.13	83.1	8.12	27.92	0.31	1.35	1.19	3.26
Hypnea japonica[c]	45.4	19.40	85.0	8.37	31.54	0.35	1.67	1.26	3.79
Brown									
Sargassum hemiphyllum (oven-dried)[d]	9.5	5.33	85.0	2.28	31.96	0.12	1.11	0.33	4.48
Sargassum hemiphyllum (freeze-dried)[d]	7.8	5.03	75.6	2.28	28.20	0.12	0.86	0.33	3.85
Sargassum henslowianum (oven-dried)[d]	33.1	11.33	86.3	3.80	31.50	0.20	0.70	0.68	3.82
Sargassum henslowianum (freeze-dried)[d]	27.0	11.93	76.9	3.80	27.99	0.20	0.63	0.68	4.08
Sargassum patens (oven-dried)[d]	48.0	7.53	84.4	2.85	33.51	0.14	0.79	0.46	4.88
Sargassum patens (freeze-dried)[d]	37.8	8.20	75.0	2.85	27.68	0.14	0.71	0.46	4.25
Green									
Ulva lactuca[c]	36.4	7.13	76.3	4.89	29.83	0.23	0.47	0.56	3.54
Other protein sources									
Soybean meal[e]	–	48.00	–	22.34	–	0.70	–	2.24	–
Fishmeal[e]	–	68.70	–	31.39	–	2.08	–	6.21	–
Spirulina[e]	–	57.50	–	27.77	–	1.15	–	3.03	–

Whole = Whole seaweed biomass as dry weight.
PE = Protein extract of seaweed.
[a] % of total protein.
[b] Data based on the mean from this review.
[c] Wong and Cheung (2001b).
[d] Wong and Cheung (2001a).
[e] NRC (2011).

(Fleurence et al., 1995; Jordan & Vilter, 1991; Wong & Cheung, 2001b). Chemical binding between protein and compounds such as polysaccharides and phenolic compounds limits the solubility of protein and reduces the yield of the soluble protein fraction (Harnedy & FitzGerald, 2011; Jordan & Vilter, 1991; Loomis & Battaile, 1966). Nonetheless, yields of 36.1–48.0% of total protein have been obtained using an initial aqueous extraction followed by an alkaline extraction (Kandasamy et al., 2012; Kumar et al., 2014; Wong & Cheung, 2001a, 2001b). However, these yields are considerably lower than those reported for terrestrial plant sources such as rice (97.4% - Ju, Hettiarachchy, and Rath (2001)) which implies that extraction protocols are not yet optimised for seaweeds. For example, seaweed extraction protocols have only focused on procedures that use dry, milled biomass based on those for terrestrial seed crops such as soybean (Berk, 1992), rice (Agboola, Ng, & Mills, 2005; Ju et al., 2001) and canola (Tan, Mailer, Blanchard, & Agboola, 2011). Terrestrial seed crops generally have relatively low concentrations of insoluble polysaccharides and most of the protein is in the form of storage proteins. In many ways seaweeds are more physiologically and biochemically similar to grasses and leaves with high concentrations of insoluble structural polysaccharides and a diverse range of proteins, many of which are associated with chloroplasts and photosynthesis, such as the enzyme RuBisCO. RuBisCO alone can represent up to 65% of total soluble leaf protein (Ellis, 1979; Spreitzer & Salvucci, 2002). The potential therefore exists to optimise protein extraction procedures for seaweeds by incorporating elements of grass and leaf protein extraction protocols that use fresh biomass and have a more mechanical focus (Bals & Dale, 2011; Chiesa & Gnansounou, 2011; Sinclair, 2009).

The crude protein (determined using a 6.25 N-to-protein conversion factor) of seaweed protein extracts has been determined in a limited number of studies (range = 33.4–86.3% dw) (Kandasamy et al., 2012; Kumar et al., 2014; Wong & Cheung, 2001a, 2001b). If we calculate the concentration of essential amino acids as a proportion of the extract (for those studies that also determined the quality of protein – amino acids as a proportion of protein), we find that the total essential amino acids, methionine and lysine increase substantially in protein extracts compared to the whole seaweed (Table 3). Protein extracts have between 3.4 and 14.0 times more total essential amino acids, 2.0–9.5 times more methionine and 2.7–13.6 times more lysine than seaweed on a whole weight basis. These extracts have higher concentrations of total essential amino acids and lysine than soybean meal, and, similar concentrations of total essential amino acids to fishmeal, although lower concentrations of lysine than the latter. In contrast, the concentration of methionine does not increase to the same extent and is similar to that of soybean meal. Notably, the other sulfur-containing amino acid cysteine (which spares methionine in nutrition) was absent from all extracts, suggesting that it may be destroyed by the high pH of the extraction process (Berk, 1992).

In addition to the high concentrations of essential amino acids, seaweed protein extracts also have high *in vitro* digestibility (Wong & Cheung, 2001a, 2001b) and functional properties (emulsifying and foaming properties and water- and oil-holding capacities) that are comparable to other protein concentrates (Kandasamy et al., 2012; Kumar et al., 2014). Moreover, seaweed protein extracts have comparable effects to casein controls on the growth and health parameters in rats (Wong, Cheung, & Ang, 2004). These qualities are strong indicators that seaweed protein extracts can be used more broadly as a protein source in the compound diets of mono-gastric livestock.

6. Future research

Seaweeds provide an opportunity to supply a novel source of protein for mono-gastric livestock but only through the concentration of protein from carefully selected species. Notably, the commercially produced red seaweeds *Gracilaria* and *Pyropia*, brown seaweeds *Hizikia* and *Laminaria* (*Saccharina*) and green seaweed *Ulva* (Mata et al., 2016) all have a high quality of protein (Table S5). These seaweeds also have relatively high concentrations of essential amino acids on a whole biomass basis (see Table S5). There is also the possibility to complement the traditional methods of protein concentration and extraction (Fleurence et al., 1995; Ju et al., 2001; Wong & Cheung, 2001a, 2001b) with industrial processing procedures employed for grasses and leaves. This research should focus on optimising the yields (proportion of total protein extracted) and the concentration of essential amino acids in the extract or concentrate (as a % of dw), and examine the functional properties, toxicity, digestibility, and performance in *in vivo* growth trials.

7. Conclusion

The quality of protein in seaweeds, including that of the commercially-available species, is comparable to or better than that of the traditional protein sources of soybean or fishmeal. Seaweeds generally contain more total essential amino acids and methionine as a proportion of protein than soybean meal, the most widely used protein source. However, the concentration of essential amino acids in seaweeds on a whole biomass basis is considerably lower than traditional sources and is not adequate as a protein component of compound diets for mono-gastric livestock. This does not detract from their positive health benefits to humans (Fleurence et al., 2012; Holdt & Kraan, 2011; Mabeau & Fleurence, 1993) and livestock (at low inclusion levels - Dierick, Ovyn, and De Smet (2009), Katayama et al. (2011)), where a low calorific value and high mineral content can be desirable. If seaweed protein is to be used for commercial mono-gastric livestock production it needs to be concentrated by the removal of fibre and ash components, or selectively extracted so that the concentration of essential amino acids can be increased to comparable levels with other protein products. This may ultimately be a stepping stone for the processing of seaweed protein to enter the targeted human supplement market.

Acknowledgements

This research is part of the MBD Energy Research and Development program for Biological Carbon Capture and Storage and is supported by the Australian Government through the Australian Renewable Energy Agency (ARENA 002369), and the Australian Government's Cooperative Research Centre Scheme (DP109602).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tifs.2016.05.014.

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