INSECT-BASED PROTEIN NUTRITION IN THE AQUACULTURE SECTOR: POTENTIAL, CURRENT SITUATION, AND CHALLENGES

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SUMMARY

Consumption of insect protein as food as well as its use in animal feed has become a trend. The animal nutrition industry became more receptive to this trend following a publication by FAO in 2013 encouraging the use of insect protein as food and feed base on its nutrient content and environmental advantages. The trend is particularly receiving a lot of attention in animal nutrition because the current protein source of the highest quality which is fish meal, is unsustainable, expensive and in high demand. Insect protein exhibits high potential due to its protein content (comparable to soy meal and fishmeal), amino acid profile, lipid content, vitamins and mineral. The potential of insect protein is not limited to its nutritional content; it also has a low environmental impact as well as the potential to convert waste to protein mass. It has great potential in aquaculture as it forms part of the diets of fish in their natural habitat, insect protein feed is especially of great advantage in the feeding of carnivore fishes. The potential of insect protein as functional food has also been highlighted by some researches as it is shown to have an immunostimulant effect which related to the presence of chitin. The use of insect has not been without its challenges such as safety concerns, poor absorption of fat and bioavailability of protein due to the presence of high chitin content. There are also issues related to the development of an appropriate processing method. This review examines the various potentials of the use of insect protein it aquaculture, the result of insect protein feeding in different species and the challenges of the use of insect protein.

ÖSSZEFOGLALÁS

Toviho, O. A. – Kovács, L. – Bársony, P.: ROVARFEHÉRJE-ALAPÚ TAKARMÁNYOZÁS AZ AKVAKULTÚRA-ÁGAZATBAN: LEHETŐSÉGEK, JELENLEGI HELYZET ÉS KIHÍVÁSOK

A rovarfehérje táplálékként való fogyasztása, valamint az állatok takarmányozásában való felhasználása trenddé vált. A tendenciát a *FAO* 2013. évi kiadványa ösztönözte, amely a rovarfehérjék táplálkozási és környezeti előnyeit ecsetelte. A rovarfehérjében lévő lehetőségek különösen nagy figyelmet keltettek a takarmányozás területén, hiszen a jelenlegi legjobb minőségű állati eredetű fehérjeforrásnak, a hallisztnek ilyen szintű felhasználása drága és gyakorlatilag fenntarthatatlan. A rovarfehérje felhasználása jelentős lehet fehérjetartalma (összehasonlítva a szójadarával és a halliszttel), az aminosav-összetétele, a zsír-, a vitamin- és ásványianyag-tartalma miatt. A rovarfehérje felhasználásában rejlő lehetőség nem csak és kizárólag a tápanyagtartalma miatt érdekes, hanem kisebb a környezetre gyakorolt negatív hatása. További szempont, hogy a szerves hulladékot használható fehérjévé lehet alakítani. Felhasználása meghatározó jelentőségű lehet az akvakultúrában, mivel a rovarok egyébként is a halak táplálékának részét képezi természetes élőhelyükben. Így a rovarfehérjére alapozott takarmányoknak különösen nagy előnye lehet a ragadozóhalak takarmányozásában. A rovarfehérje funkcionális táplálékként is használható immunstimuláló hatása következtében, amely a kitin jelenlétéhez kapcsolódik. Ennek ellenére a rovarfehérje szélesebb körű használatához néhány problémát még meg kell oldani. Számos biztonsági aggály merül fel vele kapcsolatban, a zsírok relatív rossz felszívódása és a fehérje korlátozott emészthetősége a magas kitintartalom miatt. A megfelelő feldolgozási módszer kifejlesztése is az elkövetkezendő évek feladata. Jelen közlemény áttekinti az akvakultúrában használt rovarfehérjék felhasználásának különféle lehetőségeit, a rovarfehérjék etetésének eredményét különböző halfajok esetében, valamint a rovarfehérjék alkalmazásának kihívásait.

INTRODUCTION

The development of commercial aquatic feeds has traditionally been dependent on the fish meal (FM) as the main protein source (*Henry et al.,* 2015). Due to the decrease in the availability and the increase in the prices of FM the search for sustainable alternatives have been prompted (*Wang et al.,* 2019).

Using insect meal instead of fishmeal is becoming increasingly common in the aquaculture sector of many countries. Besides the fact that fishmeal is not eco-friendly as a principal dietary protein source, it is also becoming costlier. Arising issues like increasing global demand for fish protein, the impact of fishmeal production on the ecology of fishing grounds, its shortage and its high price have brought attention to the need for alternative dietary protein sources (*FAO,* 2016, 2014). Animal and non-animal protein from legume and/or oil seeds or cereal gluten are now used as substitutes (*FAO*, 2014; *Tran et al.,* 2015). Regrettably, plant protein derivatives rarely have a balanced essential amino acid (EAA) profile, and often contain antinutritive factors, are limited in palatability and have high proportions of fibre and non-starch polysaccharides (*Sanchez-Muros et al.,* 2014; *Oliva-Teles et al.,* 2015; *Tran et al.,* 2015). Processed animal protein is considered a valuable alternative as it has a better EAA profile and is more digestible than plant proteins; nevertheless, within the Europe Community, restrictions on the use of certain processed animal proteins persist as a preventive measure against the transmission of spongiform encephalopathies (*Regulation 68/2013/EC*, 2013) (*Bruni et al.,* 2018).

Plant feedstuffs are usually deficient in protein contents, essential amino acid balances, presence of anti-nutritional factors and absent of certain FM components (i.e. taurine and hydroxyproline) leading to the potential problems of poor growth performance, intestinal inflammation and decreased palatability. Over the last decade, the value of insect protein as partial or complete replacements for FM has been studied (*Wang et al.,* 2019). The European Commission has recently approved the use of processed insect protein in aquafeeds (*Regulation 2017/893/EC*, 2017) (*Bruni et al.,* 2018; *Wang et al.,* 2019). The recent EU commission regulation (2017/893-24/05/2017) authorized the use of 7 insects (2 flies, 2 mealworms, 3 cricket species) in aquafeeds, this will further motivate the intensification of their production (*Henry et al.,* 2018).

The replacement of expensive and unsustainable fish meal (FM) and fish oil in aquafeeds has already received a lot of attention for finfish production to ensure increased profitable and sustainable. Researches have however focused on commodities such as oilseeds (especially soybeans), meat by-products (such as blood meal and bone meal) and microbial proteins. Complete replacement of FM and fish oil in finfish aquaculture feeds has been meeting with several drawbacks. Particularly for carnivorous fish, vegetable proteins have inappropriate amino-acid balance, poor protein digestibility and anti-nutritional substances. This necessitates research into the inclusion of other highly nutritious supplements such as microalgae and/or meat by-products. It is, however, important to note that these ingredients do not always meet the expected ecological, nutritional and economical requirements. As a consequence, both finfish and ornamental aquaculture could benefit from alternatives that ensure fish health and welfare standards by providing proper feeding stimulants, proper levels of essential amino acids and polyunsaturated fatty acids (PUFAs), high nutrient and energy bioavailability, as well as reduced anti-nutritional factors. The use of insect meal instead of fishmeal is becoming more common in the aquaculture sector of many countries. Fishmeal is not eco-friendly as a principal dietary protein source; it is also not cost-effective as the price continues to increase. Animal and fishery by-products and plant-derived material are now used as substitutes (*FAO*, 2014). The increasing attention attracted recently by insects as a sustainable nutrient source for feed is not only in Europe but also around the world. Insects are a good source of EAA, lipids, vitamins and minerals (*van Huis et al.,* 2013; *Henry et al.,* 2015); they grow and reproduce quickly and easily on low-quality organic waste and manure (*van Huis et al.,* 2013); they have a small ecological footprint and high feed conversion efficiency (*Makkar et al.,* 2014), and can reasonably foster a circular bioeconomy (*Bruni et al.,* 2018). The protein content of most insect species is around 60%, this value can vary between 7% and 91% (dry weight) (*Fasolin et al 2019).* One of the most intensively investigated species for fish feed production *is Hermetia illucens* (*Diptera: Stratiomyidae*) or Black Soldier Fly (BSF) (*Révész and Biró,* 2019). The variations in nutrient composition of BSF, i.e. crude protein and crude lipid can vary from 40% to 54% and 15% to 49%, based on the feeding substrate and processing methods (*Wang et al.,* 2019). Yellow mealworm, *Tenebrio molitor* (TM), is another insect commercially produced to be used as pet food (birds and reptiles) or fishing baits, they have a protein content of 47-60%; up to 70% in defatted meal and lipids (31%-43%) and their amino acids and fatty acids profiles are suitable for inclusion in animal feeds (*Henry et al.,* 2018).

Fish farming, or aquaculture, is expected to amount to 62% of the global fish supply by 2030. This translates to increased demand for fish meal and fish oil to feed farmed fish. The changing climatic conditions in Peru has adversely affected the availability of fish meal and fish oil, causing a decrease in availability and high volatility on the market. Processed animal proteins (PAPs) which are allowed in fish feed production, are yet to be included in many of the feed products on the market today. Insect protein has similar characteristics to PAPs and provides a good, sustainable alternative (*IPIFF,* 2018).

In 2014, about 10% of world fish production (captured and aquaculture) went into fish meal and fish oil production. Fish meal is made from small wild-caught marine fish that contain a high percentage of bones and oil, and are usually deemed unsuitable for direct human consumption. Fish meal is a high-quality feed ingredient for pigs, poultry, and aquaculture and is used extensively (*van Huis and Oonincx,* 2017). Between 1988 and 2010, the poultry sector decreased the use of fish meal from 60% to 12% while the aquaculture sector increased its use of fish meal from 10% to 56% in the same period. Increasing fish meal prices have led to lower inclusion percentages in aquafeed, however, the effect is not evident on fish meal use as it is quickly offset by the rapid growth of the aquaculture sector (*Olsen and Hasan* 2012; *Msangi et al*., 2013). This has necessitated the search for alternative sources, for instance, the use of plant material (*van Huis and Oonincx,* 2017).

POTENTIAL

Ninety-five percent of the animal kingdom consists of insects, as this group is extremely diverse. Using insects as compared to the existing protein sources has several advantages, such as fewer greenhouse gas emissions, less water and land used, and higher efficiency of protein conversion (*Yoon et al.,* 2019).

Insects are a natural component of the diets of carnivorous fish, poultry and pigs (*IPIFF*, 2018). The use of alternative ingredients in aquafeeds is intended to reduce the dependence on scarce, expensive or unsustainable feedstuff. Insect meal stands out as an alternative ingredient to be included in animal feeds. Insects are a natural food source for marine and freshwater fish species (*Fontes et al.,* 2019).

They have high protein content from 50% to 82% dry matter and can be added to animal feed with up to 40% insect content for fish feed and 30% for chicken feed. Insect products have an amino acid profile that makes them highly digestible for animals. The amino acid profiles of most insect species that have been tested in farmed fish feed formula have a good correlation with the fish's specific needs. Insects have also been shown to promote nutrient uptake and show promising results in animal growth performance. This upholds their use as complementary source material in feed formulation for aquaculture (*IPIFF,* 2018). Another factor that has drawn attention to the use of insect protein as a feed ingredient in fish feed is its immunostimulation activity (*Ido et al.,* 2019). Results of preliminary studies have shown that certain bioactive insect components led to improved immunity and reduced mortality rates when in aquaculture feed e.g. for shrimp and salmon. Prebiotic fibres like chitin serve to provide nutrients for probiotic gut bacteria. Roughly 1,000 tonnes of insect protein have been commercialised by European insect producers since the authorisation of insect proteins for use in aquafeed. And the aquafeed market has consumed approximately 50% of European animal feed produced from insect protein and this is statistic is expected to rise to 75% by 2030. Presently, one-third of all food is wasted. Measures are being taken to create a healthier, more sustainable food production and consumption system which produces less waste (*IPIFF,* 2018). To achieve that goal, the European Commission launched the Food 2030 research and innovation policy which responds to the UN Sustainable Development Goals (SDGs). SDG 12, 'Ensure Sustainable Consumption and Production Patterns', is of relevance for the insect sector. Insects are feed with co-products from the cereal, starch, fruit and vegetable supply chains or local food processors e.g. pastry and biscuits, local artisans e.g. bakers or unsold products from supermarkets which are unsold for technical or logistical reasons. By turning lower-value materials and ingredients with low environmental footprints into high-value materials, such as proteins, insect producers offer a new outlet and a sustainable alternative for unexploited or underexploited resources, in accordance with the waste hierarchy principles (*IPIFF,* 2018).

CURRENT SITUATION

The application of various insect species as a replacement for fish meal in aquaculture has been shown in different feeding trials focusing on several commercially viable species (*Tschirner and Kloas* 2017). According to Fishstat by

FAO, the important species of fish by the quantity of production in Europe from the highest to the lowest are atlantic salmon, rainbow trout, gilthead sea bream and European seabass, all of which have been used in researching the effect of replacing fishmeal with insect meal. Several studies have evaluated the use of insect protein in the feed of freshwater species, such as African catfish (*Clarias gariepinus*), rainbow trout (*Oncorhynchus mykiss*), Jian carp (*Cyprinus carpio)*, yellow catfish (*Pelteobagrus fulvidraco*), red tilapia (*Oreochromis* sp.), Nile tilapia (*Oreochromis niloticus*) (*Fontes et al.,* 2019), giant freshwater prawn (*Macrobrachium rosenbergii*) (*Feng et al.,* 2019) and some salt water species like; Pacific White Shrimp (*Choi et al.,* 2018; *Motte et al.,* 2019). Positive results have been reported in aquaculture species fed insect meal in their diet (*Bandara*, 2018). Among the positive results reported includes, improved weight gain (*Choi et al.,* 2018; *Motte et al.,* 2019), better SGR (specific growth rate) (*Choi et al.,* 2018), better FCR (feed conversion ratio) (*Motte et al.,* 2019), increased lipid content (*Panini et al.,* 2017) immune-modulation (*Yixiang et al.,* 2013; *Ido et al.,* 2015; *Choi et al.,* 2018; *Ido et al.,* 2019; *Motte et al.,* 2019).

Insect Meal Use in Fish Culture
Effect of insect meal on growth performance of fish

Defatted Black soldier fly larvae meal (DBSFLM) has been shown as a promising fish meal substitute in diets for Turbot (*Scophthalmus maximus*), Rainbow trout (*Oncorhynchus mykiss*), Jian carp (*Cyprinus carpio 'jian'*), Pacific white shrimp (*Litopenaeus vannamei*) and Atlantic salmon (*Salmo salar*). *Wang et al.* (2019) examined DBSFLM as an alternative protein source in Japanese seabass (*Lateolabrax japonicus*) diets. Five diets were formulated by replacing FM (0%, 16%, 32%, 48% and 64%). Results showed that growth performance, somatic indexes, hepatic and intestinal histomorphology, and the intestinal antioxidant and immunity indexes of fish were not affected by dietary treatments. At 48% and 64% insect meal inclusion there was higher feed intake, but lower whole body ash content and ash retention, lower serum concentrations of total cholesterol, triacylglycerol, high density lipoprotein cholesterol and malondialdehyde (MDA) than that fed FM. Inclusion of insect meal did not alter activities of hepatic trypsin, lipase and amylase, but increased activity of intestinal lipase for fish fed 48% and 64% insect meal than that fed FM (*Wang et al.,* 2019).

The nutritional value and energy apparent digestibility coefficient (ADC) of five insects for Nile Tilapia male fingerlings were examined by formulating six dietary treatments; (control, *Nauphoeta cinerea* meal (NCM), *Zophobas morio* larvae meal (ZMM), *Gromphadorhina portentosa* meal (GPM), *Gryllus assimilis* meal (GAM) and *Tenebrio molitor* larvae meal (TMM)). The control diet had no insect meal included while the other five treatments comprised 80% commercial diet and 20% test ingredient. TMM presented a higher ADC for dry matter, protein, corrected protein and chitin than to other treatments. GPM presented the highest ADC for lipids. The outcome of the study is that, the TMM presented better ADC of nutrients and energy; furthermore, all the insect meals evaluated are potential feed for Nile tilapia fingerlings (*Fontes et al.,* 2019)

Rema et al. (2019) assessed the effect of incorporation levels of defatted yellow mealworm (*Tenebrio molitor*) protein meal on growth performance, body composition, and apparent nutrient digestibility of juvenile rainbow trout (*Oncorhynchus mykiss*). There were five experimental diets: control diet with 25% fishmeal, and four experimental diets with yellow mealworm protein with fishmeal replacement of 20%, 30%, 60%, or 100%, respectively. At the end of the experiment, there was a significant stepwise increase in final body weight, and a significant improvement of specific growth rate, feed conversion ratio, and protein efficiency ratio in comparison to the control treatment. At all inclusion level of insect protein, there was no effect on the fish whole-body composition and apparent digestibility coefficients of dry matter, protein, fat, phosphorus, and energy. Protein, phosphorus, and energy retention significantly increased in fish fed insect protein meal diets. The study concluded that the yellow mealworm protein meal could effectively replace 100% of fishmeal in the diet of juvenile rainbow trout with positive effects on its overall zootechnical performance. It is particularly important to highlight that the best growth performance and FCR were recorded when FM was fully replaced by IPM, supporting that this IPM is a sustainable and environmentally friendly replacement for expensive and unsustainable fish meal in the diet of rainbow trout (*Rema et al.,* 2019).

Studies conducted with Atlantic salmon showed that fish meal can be completely replaced by *Hermetia illucens* in their diet without adverse effects on net growth of the fish, histology, odour, flavour/taste, and texture (*Lock et al.*, 2016). Similarly, the meal made from the black soldier fly is a suitable protein source for many other farmed fish species, such as African catfish (*Clarias gariepinus)* (*Adeniyi and Folorunsho* 2015; *Anvo et al.*, 2016), Channel catfish (*Ictalurus punctatus*), and Blue tilapia (*Oreochromis aureus*) (*Bondari and Sheppard* 1987). Yellow mealworm meal could also replace up to 35% fish meal in the diet of European sea bass (*Dicentrarchus labrax*) without affecting mortality or growth (*Gasco et al.,* 2016). A similar trial conducted with Rainbow trout (*Oncorhynchus mykiss*) showed that weight gain was not affected at higher inclusion levels of mealworm meal, while the protein content increased and lipid contents of fillets decreased, compared to the control (*Belforti et al.,* 2015; *van Huis and Oonincx,* 2017).

Immunomodulatory effect of insect meal on fish

Studies have indicated the protective effect of dietary insects, and it was suggested to be either directly through the secretion of antimicrobial peptides by the insect, indirect through the stimulation of the fish immune system by chitin (*Esteban et al.,* 2000; *Esteban et al.,* 2001; *Lee et al.,* 2008) or by other insect components (*Ido et al.,* 2015). The first hypothesis involving the secretion of antimicrobial peptides by the insects seemed however unlikely as dead insects do not secrete any AMPs. The indirect effect through the immunostimulation of the fish was therefore, more likely (*Henry et al,* 2018).

European sea bass (*Dicentrarchus labrax*) fed with *Tenebrio molitor* larval meal for 6 weeks exhibited significant anti-inflammatory responses. The number of studies on the effect of insect meal on the immune system and antioxidant enzymes of the fish is increasing fast. Black carp (*Mylopharyngodon piceus*) fed with low doses (2.5%) of maggot (*Musca domestica*) for 60 days showed increased serum lysozyme, serum complement and liver superoxide dismutase (SOD) and catalase (CAT) activities and reduced liver MDA suggesting an increased anti-bacterial activity and antioxidant activity of the insect meal at a low dietary dose (*Yixiang, et al.,* 2013). The insect meal also protected these fish against a bacterial challenge with *Aeromonas hydrophila* (*Ming et al.,* 2013). A similar study in Red seabream *(Pagrus major)* showed that the introduction of low doses 0.75% and 7.5% of Housefly (*Musca domestica*) pupae in the diet of red sea bream for 10 days showed a significant increase of the phagocytic activity of peritoneal macrophages (*Ido et al.,* 2015). Interestingly, 5% of dietary housefly pupae for 2 months protected (100% survival) the fish against the bacterial pathogen *Edwardsiella tarda* while all control fish died 12 days after the bacterial challenge (*Ido et al.,* 2015). A low inclusion of housefly (*Musca domestica*) pupae has also been reported to increase phagocytic activity and disease resistance against *Edwardsiella tarda* in red seabream (*Pargus major*). Dietary inclusion of MW is also known to increase the enzyme activities of the immune systems of European seabass, rainbow trout, mandarin fish (*Siniperca scherzeri*), and pearl gentian grouper (*Ido et al.,* 2019).

Effect of Insect Meal in Shrimp Culture
Effect of insect meal use on growth of shrimp

Experimental diets were formulated to replace fish meal with mealworm (*Tenebrio molitor*) at a ratio of 25%, 50%, and 100% for Pacific white shrimp (*Litopenaeus vannamei*). The final weight, weight gain, and SGR values of shrimp fed mealworm diets improved significantly in comparison to the shrimps fed the control diet. The shrimps feed mealworm meal at 50% inclusion level had the highest growth performance, indicating that 50% inclusion of mealworm could be optimal to replace fish meal in the diet of *L. vannamei* (*Choi et al.,* 2018). Similarly, another study compared the growth and immune parameters of juvenile Pacific white shrimp fed experimental diets where FM was replaced with an insect meal after an eight-week feeding trial. Four diets in which a proportion of FM was replaced by yellow mealworm (YM 25%, 50%, 75%, and 100%) were formulated with a control diet with no insect meal. All diets were isoproteinic, isoenergetic, and balanced in lysine and methionine. Growth and feed conversion parameters improved when YM was included in shrimp diets; with the highest weight gain and best feed conversion ratio (FCR) achieved when 50% of FM was replaced by YM versus the control diet that contained no YM (initial weight: 1.60 g/shrimp; growth: 5.27 g/shrimp vs. 3.94 g/shrimp; FCR 1.20 vs. 1.59). Replacing FM with YM improved all of the growth performance parameters of shrimp. When compared with the control diet based on FM only, specific growth rates were significantly higher among shrimp that were fed diets in which 25%, 50%, and 100% of FM were replaced with YM. Moreover, replacing from 25% to 75% of FM with YM led to significant increases in weight gain (*Motte et al.,* 2019). According to *Panini et al.* (2017) weight gain, specific growth rate, feed intake, feed conversion, survival, and protein retention of pacific white shrimp were not affected by replacing fishmeal with mealworm meal (MM). There were no significant differences in protein content between the treatments. But, the moisture content showed a linear decrease with an increase in the fishmeal replacement level while the lipid content increased with MM inclusion level (*Panini et al.,* 2017). Four diets were formulated with 4% (D4), 8% (D8), 12% (D12), and 16% (D16) *Tenebrio molitor* protein and compared with a control to investigate the effects of *T. molitor* protein on growth performance, immunological parameters, and resistance against *Lactococcus garvieae* and *Aeromonas hydrophila* in *Macrobrachium rosenbergii*. After 10 weeks of the experiment, the study found that weight gain (WG), percentage of weight gain (PWG), daily growth rate (DGR), specific growth rate (SGR), and protein efficiency ratio (PER) of D12 were significantly higher than those of the control, D4, and D8, but not significantly different from those of D16. The condition factor (K), feed conversion ratio (FCR), and survival rate (SR) did not differ significantly among groups. Lipid content decreased and protein content increased in the carcass and muscle of prawns as *T. molitor* protein contents increased (*Feng et al.,* 2019).

Immune-modulatory effect of insect meal uses on shrimp

Experimental diets formulated to replace a fish meal with mealworm (*Tenebrio molitor*) at a ratio of 25%, 50%, and 100% for Pacific white shrimp showed a protective effect against WSSV (white spot syndrome virus) infection. After 3 days WSSV challenge, the mortalities of groups fed Control, MW25, MW50 and MW100 were 100%, 40%, 30%, and 70%, respectively which is an indication of enhanced immune modulation in groups fed insect protein. Increased total haemolymph count (THC) indicates that mealworm based diet could improve the immunity of shrimps (*Choi et al.,* 2018). *Motte et al.* (2019), in their study also challenged shrimp feed diets containing yellow mealworm meal with pathogenic bacteria (*Vibrio parahaemolyticus*). They found that in challenged shrimp, mortality rates were significantly less among groups fed yellow meal worm meal, with a 76.9% lower mortality rate in the 50% FM replacement group versus the control. Shrimps do not have an adaptive immune system to fight diseases making the health of shrimp and the enhancement of the innate immune system are of primary concern. Intensive shrimp producers have to find ways to boost the innate immune system of shrimp to improve disease resistance. Dietary chitin and krill (chitin-rich) have been shown to modulate the immune system of fish and shrimp (*Motte et al.,* 2019).

CHALLENGES

The European approach to insects as feed is limited to a large extent by the issue of Bovine Spongiform Encephalopathy (BSE), which poses a serious threat to consumer health and safety. The first ban on the use of processed animal proteins (PAPs) was in 2001, due to transmissible BSE regulation. This led to the Directive 2002/32 which established the materials intended for use in animal feed, undesirable substances and their maximum allowable levels in feed. After the directive successive element included in feed must comply with the limits on undesirable substances. After a few years, the PAPs prohibition was amended and Regulation 56/2013 led to the use of PAPs from any source, except ruminants, in aquaculture. Nevertheless, insects were not yet specifically regulated in the raw material catalogue (Regulation (UE) 68/2013).

Regulation (EU) 2017/893 authorizes the feeding of non-ruminant processed animal protein to aquaculture animals only. Annex II lists processed animal protein derived from farmed insects intended for the production of feed for farmed animals other than fur animals. So far, only seven species are permitted for use: the house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*), field cricket (*Gryllus assimilis*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), black soldier fly (*Hermetia illucens*), and the common house fly (*Musca domestica*); the regulation also specifies the substrates allowed as feed for insects.

In 2017, an amendment was made to Regulation (EU) No. 68/2013 for the catalogue of feed materials, Regulation (EU) 2017/1017 permitted the use of live terrestrial invertebrates and dead terrestrial invertebrates with or without treatment as feed materials, but not as processed as described in Regulation (EC) No. 1069/2009. As a result, terrestrial invertebrates are now considered appropriate materials for feed in all stages of their life cycle, except species having adverse effects on plant, animal, or human health (*Sogari et al.,* 2019).

Chitin may interfere with the dietary utilization of protein, where a reduction in protein digestibility due to an increase in the chitin content is expected. The type of chitin matrix in insects may have a negative effect on chitinase activity and thus protein digestibility (*Fontes et al.,* 2019). The efficiency of chitin utilization by monogastric animals is usually discussed about the presence, or lack of, chitinolytic enzymes; however, some studies have confirmed the presence of chitinolytic enzymes in various organs of fish species, such as the gastric mucosa, intestinal mucosa, pyloric caeca and pancreas. According to a previous study, chitin may inhibit the absorption of lipids in the gut, increasing their excretion and, thereby adversely impacting on their digestibility coefficient. Although the insect meal chitin content ranged from 12.01% to 28.94%, the study by *Fontes et al.* (2019), show that chitin did not seem to influence lipid digestibility, probably due to its high digestibility. The *G. portentosa* meal presented the highest chitin content (*Fontes et al.,* 2019)

The presence of chitin in aquafeeds is likely to induce a general reduction of the diet digestibility. The effect of the chitin in fish feeds is yet to be fully understood, as results there are controversies. Some previous studies have shown that moderate inclusion of chitin resulted in increased fish immune response and positive microbiota modulation while others reported the negative effects for example; possible intestinal inflammation and reduced nutrient digestibility and assimilation (*Vargas-Abúndeza et al.,* 2019). More recent studies however show no signs of severe intestinal inflammation by the histological analyses in all the samples analysed, except for a reduction in intestinal fold length which was exclusively observed in fish fed 50% and 75% *Hermetia illucens* meal substitution diets (*Vargas-Abúndeza et al.,* 2019). Shortening of intestinal folds have previously been associated with impaired nutrient absorption which ultimately translates to growth reduction (*Moldal et al.,* 2014); however, in this study this was not observed and the GR and HSP70 molecular markers involved in stress response (these markers are useful for detecting stress; inflammation, physiological responses) showed no significant difference among groups, suggesting general fish welfare. Insect fatty acid composition could be the reason for the absence of intestine inflammation (*Vargas-Abúndeza et al.,* 2019).

Moreover, the production, marketing and use of edible insects as food and feed cut across a wide range of regulatory institutions, whose obligation is to ensure aspects such as the quality and safety of the products obtained and the environmental impact of insect breeding. It is very difficult to produce a single protocol on the processing of insect proteins, because each species is peculiar in size, culture and reproduction, stages of life, protein content and digestibility and availability of amino acids (*Lucas et al.,* 2019).

A crucial aspect that has not got a lot of attention with regards to research is the safety of insect meal for feed production. Bacteria and viruses such densovirus in *Acheta domesticus* and *Gryllodus* species, cricket paralysis virus in crickets, fungi (*Fusarium*, *Cladosporium* species) have been found in insects. Although the available information from research shows that most viruses which are taxonomically related to insect are unable to replicate in the insect and as a result of this pose no health concern, the burden related to virus of insect are carried by the insect farm. However, there is a group of virus that can replicate in vector; the arboviruses. There is a need to research this group of virus (*Van et al.,* 2018).

Data relating to the microbiology of insects and their potential pathogens are mainly available in studies that consider insects as pests rather than food animals (*Belluco et al.,* 2013). In these cases, insects were investigated for their potential to act as vectors for foodborne pathogens in farming conditions. Such data are of limited value in the context of farms rearing insects fit for human consumption; however, they provide some qualitative information (*Belluco et al.,* 2013). Few data are available to support risk analysis, particularly for the use of insect as feed. Only isolated information related to the chemical risk of insect is available in publication regarding food and feed made (*Charlton et al.,* 2015).

There are endogenous risk factors in insect like antinutrient substances and allergens. It was determined that the pupae of the African silkworm *Anaphe* spp contain heat-resistant thiaminase which is responsible for seasonal ataxic syndrome cases due to thiamine deficiency in Nigeria for the last 40 years. Furthermore, it has been discovered that insects just like other arthropods (e.g., shellfish) can cause allergic reactions (*Rumpold and Schlüter*, 2013).

CONCLUSION

The insect could easily be the sustainable protein source of the future based on FCE and low environmental footprint. It could play a large role as an alternative protein source in contributing to the reduction of world hunger. Insect as a protein source has a lot of potential in the aquaculture industry, it is more practical in aquaculture as an insect serves as a part of the diet of fish in their natural environment. It is however important that care should be taken and further research carried out because most of the qualities that are desirable in insect as a protein source cannot be generalized for all insect. Nutrient composition varies based on species, stage of the life cycle, and type of feed. Also, the suitability and inclusion level of insect protein in feed depend on the aquaculture species to be fed; therefore, result from the research of a species cannot be generalized for other aquaculture species. There is also the need to fill the knowledge gap based on the processing of insect protein as well as the safety concerns associated with insect protein. There is an increasing number of researches on insect protein but there is still a lot of work to be done in this area.

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