REBECA FILIPA PEREIRA ANTUNES

Replacement of soybean meal and soybean oil in pig diets with full-fat *Hermetia Illucens* larva meal – a metabolic essay

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Abstract

The projected increases in the global population with an increasing consumption of animal products are expected to add pressure on the environment and natural resources. The production of feed is resource demanding and responsible for a large part of the environmental impact of livestock production. In the search for more sustainable and alternative nutritional sources, insects stand out for their ability of converting by-products and food residues into animal biomass rich in protein and fat, with potential for application in feed.

An experiment was conducted with Pietrain × (Duroc × Large White × Landrace) crossbred pigs aiming to evaluate the effect of the partial and total replacement of soybean meal and soybean oil by full-fat black soldier fly larva meal on the metabolic characteristics and growth performance. Experimental diets (SBM- control, BSF50- 50% replacement; BSF100- 100% replacement) were formulated to be isoenergetic and isoproteic (15% CP). Metabolic trials were performed to study the experimental diets with finishing pigs. Following a randomized block experimental design twelve non castrated males, with average live body weight of 65,52 ± 2,2 kg, were individually allocated in metabolism cages for the control of feed intake and separated sampling of feces and urine, for 7 days periods, in alternate weeks all over the finishing period, after adaptation to the experimental diets. The trial lasted for 35 days. The inclusion of larva meal in diets was 9% and 18% respectively for diets BSF50 and BSF100. Live body weights (BW), average daily gain (ADG) and feed conversion ratio (FCR) were estimated weekly. Feed and water intake and excreta were measured daily. Diets and excreta were chemically analyzed and apparent total tract digestibility (ATTD) of Dry Matter (DM), Nitrogen (N) Energy (E), Calcium (C) and Phosphorus (P) and balance of N, E were calculated. Body weight gain (BWG) and average daily gain (ADG) were affected by diet. The BSF100 dietary treatment decreased the BWG and ADG. No significant effects of diet were observed for feed conversion ratio (FCR) and average daily feed intake (ADFI). There were no significant differences between the apparent digestibility (ATTD) of the experimental diets, except for the dry matter and crude energy ATTD.

The obtained results showed that a complete substitution of soybean meal for BSF meal can be done without impacting nutrient digestibility. However, further investigations on productive performance parameters should be performed.

Keywords: Growing-finishing pigs, *Hermetia Illucens* larva meal, Soybean substitution, Apparent digestibility, Growth performance

Resumo

O crescimento da população mundial acompanhado por um aumento do consumo de produtos de origem animal vai adicionar pressão ao ambiente e aos recursos naturais. A produção de alimentos para animais é muito exigente em recursos e é responsável por uma grande parte do impacto ambiental da produção pecuária. Na procura por fontes nutricionais alternativas mais sustentáveis, os insetos destacam-se pela sua capacidade de bioconversão de subprodutos e resíduos alimentares em biomassa animal rica em proteína e gordura, com potencial para aplicação na alimentação animal.

Foi realizado um estudo com o objetivo de avaliar o efeito da substituição parcial e total da farinha de soja e óleo de soja por farinha de larvas de mosca soldado negro, não desengordurada, nas características metabólicas e produtivas de suínos. As dietas experimentais (SBM-controlo, BSF50- 50% de substituição; BSF100- 100% de substituição) foram formuladas para serem isoenergéticas e isoprotéicas (15% CP). Estas dietas foram avaliadas em ensaios metabólicos com suínos de raça cruzada Pietrain × (Duroc × Large White × Landrace) em fase de engorda/acabamento. Seguindo um delineamento experimental de blocos casualizados, doze machos não castrados, com peso vivo médio de 65,52 ± 2,2 kg, foram alojados individualmente em caixas metabólicas para controlo diário do consumo de alimento e da excreção de fezes e de urinas, por períodos de 7 dias, em semanas alternadas durante todo o período de acabamento, após adaptação às dietas experimentais. O ensaio durou 35 dias. A inclusão da farinha de larvas nas dietas foi de 9% e 18%, respetivamente, para as dietas BSF50 e BSF100. Semanalmente os animais eram pesados (BW) e os ganhos médios diários (ADG) e índices de conversão (FCR) calculados. Os alimentos e as excretas foram analisados quimicamente para cálculo da digestibilidade aparente (ATTD) da Matéria Seca (DM), Azoto (N) Energia (GE), Cálcio (Ca) e Fósforo (P) e dos balanço de N e de E.

O ganho de peso vivo (BWG) e o ganho médio diário (ADG) foram afetados pela dieta. Nenhum efeito significativo da dieta foi observado para a taxa de conversão alimentar (FCR) e o consumo médio diário de ração (ADFI). Não houve diferenças significativas entre a digestibilidade aparente (ATTD) das dietas experimentais, exceto para a ATTD da matéria seca e da energia bruta.

Dos resultados obtidos concluiu-se que que a substituição da soja por farinha de inseto em dietas de suíno poderá ser uma oportunidade, embora haja necessidade de ensaios produtivos complementares ao estudo realizado.

Palavras-chave: suínos engorda/acabamento; farinha de larva *Hermetia Illucens,* substituição da farinha de soja, digestibilidade aparente, performance de crescimento

Resumo das secções em Português

1. Revisão bibliográfica

1.1 Introdução

1.1.1 O aumento do consumo de produtos de origem animal

A FAO prevê que nos próximos anos a produção de alimentos a um nível global tenha um crescimento de 60% face aos valores atuais, de modo a suprir a demanda de uma população em contínuo crescimento, mais urbanizada e com hábitos alimentares cada vez mais diversificados, incluindo um aumento no consumo de produtos de origem animal (Alexandratos & Bruinsma 2012). A origem deste aumento irá verificar-se principalmente nos países em desenvolvimento, onde tradicionalmente o consumo de produtos de origem animal era baixo, prevendo-se que vá atingir quase o dobro para o consumo de carne e que cresça um terço para o consumo de lacticínios até 2050, sendo que nos países desenvolvidos a tendência é de um crescimento menor, praticando atualmente consumos elevados (Herrero *et al.* 2015).

1.1.2 Os desafios do setor no século XXI: alterações climáticas, escassez de recursos e impacto ambiental

Agravando ao desafio de aumentar a produção de alimentos, o setor enfrenta vários desafios relacionados com a escassez de recursos naturais como a água e o solo arável, que sofrem de severos problemas de poluição e competição para outros usos, como a urbanização, usos industriais, produção de biocombustíveis, entre outros. Assim como a necessidade urgente de reduzir a emissão de gases com efeito de estufa e atenuar os efeitos das alterações climáticas com impacto na biodiversidade e afetando a produtividade e disponibilidade dos alimentos.

Estes desafios requerem uma mudança no paradigma da produção, procurando uma utilização mais eficiente dos recursos disponíveis em conjunto com uma redução do seu impacto no ambiente, conceito conhecido com a intensificação sustentável (Herrero *et al*. 2015). Ainda assim, a sustentabilidade será relevante da perspetiva do consumo. Vários estudos sugerem que uma mudança nos padrões de consumo, nomeadamente praticar um consumo modesto de produtos de origem animal e reduzir o desperdício alimentar, podem contribuir para uma redução do impacto ambiental do setor alimentar (Van Huis 2013; Schader *et al*. 2015; Herrero *et al*. 2016).

O setor agropecuário tem sido alvo de discussão em torno da sua sustentabilidade ambiental, particularmente pela sua vasta utilização de recursos naturais, contribuição para as alterações climáticas através da emissão de gases com efeito de estufa (GHG) e emissões de amónia tendo impacto na poluição dos solos e águas. Deste modo, o setor desempenha um papel fundamental na adoção de medidas mais eficientes que salvaguardam o meio ambiente e contribuem para o desenvolvimento sustentável da produção de alimentos.

Nos sistemas de produção animal a disponibilidade de alimentos de qualidade e acessíveis são uma peça fundamental, tanto do ponto de vista económico como ambiental. Atualmente a produção de alimentos para animais utiliza uma área de 560 milhões de hectares, o equivalente a 40% do solo arável a um nível global, utilizando uma parte significativa dos 200 milhões de toneladas de fertilizante aplicadas anualmente e cerca de 8% da água destinada ao consumo humano (Mottet *et al.* 2017; FAO 2018). É também responsável por quase metade das emissões de GHG pelo setor, estando estas relacionadas com a produção de fertilizantes, produção de alimentos, deposição e aplicação de estrume e emissões relacionadas com as mudanças no uso de terra (Gerber *et al.* 2013)

Mais adiante, com a intensificação da atividade pecuária, quantidades crescentes de estrume acumuladas em certas regiões são outra preocupação. Os nutrientes contidos nos alimentos, como nitrogénio e o fósforo, são muitas vezes fornecidos em excesso ou apresentam uma baixa biodisponibilidade sendo pouco digeridos e excretados nas fezes e urinas. Estes nutrientes presentes nos efluentes pecuários, sob certas condições de armazenamento e processamento levam a emissões de metano e óxido nitroso, constituindo também uma fonte de poluição das águas e solos.

Na cadeia de produção de suínos, a produção de alimentos é responsável pela maior parte (60%) das emissões de GHG, particularmente devido ao aumento da demanda por alimentos, sendo a soja um grande impulsionador. Por outro lado, o armazenamento e processamento do estrume é a segunda maior fonte de emissões (30%) de gases com efeito de estufa na suinicultura (MacLeod et al. 2013). Assim, dois desafios particularmente importantes prendem-se com o desenvolvimento de dietas mais sustentáveis e estratégias de maneio do estrume mais eficientes.

1.1.3 Produção e demanda por alimentos compostos para animais

A produção global de alimentos para animais cresce anualmente, acompanhando o aumento do consumo de produtos de origem animal. Em 2018 a produção mundial de alimentos compostos foi estimada em 1.1 biliões de toneladas, representando um aumento de 3% em relação ao ano anterior, tendo sido na sua maioria destinada á alimentação de frangos (44%), seguido pelos suínos (26%) e ruminantes (22%) (www.allaboutfeed.org).

Atualmente os principais ingredientes utilizados na formulação de rações incluem uma variedade de cereais e suplementos proteicos tais como a farinha de peixe e a farinha de soja, mas também subprodutos como grãos de secos de destilaria, polpas e melaços.

Uma das preocupações a cerca da segurança alimentar é a utilização de ingredientes na alimentação animal que poderiam ser diretamente empregues na alimentação humana, uma vez que a sua provisão através dos animais está sujeita a perdas de conversão e compete por uma área arável global limitada para a produção de alimentos. Atualmente um terço da produção de cereais está destinada á alimentação animal e prevê-se que até 2050 esta proporção constitua metade de toda a produção para suprir a demanda por produtos de origem animal (Makkar 2018).

A transição de sistemas extensivos de produção de ruminantes para sistemas intensivos de produção, particularmente de espécies monogástricas, é uma das razões para o incremento da utilização de cereais na alimentação animal. Apesar da maior eficiência dos frangos e suínos em comparação com os ruminantes (tipicamente 2 a 4kg de cereais vs 7kg por kg de carne produzida), estas espécies possuem uma capacidade reduzida de digestão de pastagem e fibras, necessitando de maiores quantidades de cereais e proteínas edíveis. De facto, a produção de carne de frango e suíno é na sua maioria proveniente de sistemas de produção intensiva nos países industrializados, e está a crescer exponencialmente nos países em desenvolvimento, como a China e a India, para responder á rápida demanda por produtos de origem animal (Steinfeld *et al*. 2006; Thornton 2010; Cao & Li 2013; Herrero *et al*. 2015).

Outra dimensão que gera uma crescente procura por cereais é a indústria dos biocombustíveis (bioetanol e o biodiesel). Ainda que, a utilização de certas colheitas para a produção de biocombustíveis resulte em subprodutos que podem ser introduzidos na alimentação animal, existem fatores como a baixa disponibilidade de nutrientes ou presença de fatores anti nutricionais que limitam a sua inclusão nas rações.

É estimado um crescimento na produção de suínos e frangos de 38% e 104%, respetivamente, o que vai implicar um aumento da demanda por fontes proteicas (IFIF online). As principais fontes proteicas são as oleaginosas, as proteínas animais processadas e subprodutos. Entre estas, a farinha de soja é mais utilizada em dietas para monogástricos devido ao seu elevado conteúdo proteico (até 50%), perfil de aminoácidos e alta digestibilidade.

Contudo, a sustentabilidade da produção e distribuição de soja tem sido alvo de uma crescente preocupação. O consumo generalizado de soja é suprido por apenas alguns países exportadores concentrados no continente americano, particularmente os Estados Unidos, Brasil e Argentina. Entre 2018 e 2019 estes 3 países em conjunto foram responsáveis por cerca de 80% da produção mundial de soja, ocupando uma área equivalente a 90 milhões de hectares (Fraanje & Garnett 2020). Ainda que a produtividade por hectare tenha vindo a aumentar, maioritariamente devido á intensificação da produção, a expansão da área de cultivo da soja permanece uma forte preocupação, particularmente se envolve a destruição de floresta e habitats naturais.

Por outro lado, a farinha de peixe é considerada o ingrediente mais nutritivo e com maior digestibilidade na formulação de dietas em aquacultura, mas também na suinicultura e avicultura em animais jovens. Contudo, a sua produção é dependente da disponibilidade de reservas e captura de peixes marinhos, que se encontram na sua maioria sob exploradas. Este facto tem vindo a resultar numa menor disponibilidade de farinha de peixe com um aumento do preço, debilitando a rentabilidade de muitas empresas na indústria da aquacultura. Assim, a inclusão da farinha de peixe tem vindo a diminuir compensada por um aumento na utilização de soja, óleos e cereais, apesar da menor digestibilidade da proteína e perfis de aminoácidos menos favoráveis destas matérias primas (Stamer 2015; Dicke 2018; Zarantoniello *et al.* 2018).

Em Portugal e na União Europa (EU), o setor enfrenta um longo cenário de escassez de fontes proteicas para alimentação animal. Esta situação foi exacerbada com a proibição do uso de proteínas animais processadas (farinha de sangue e osso) de modo a conter o surto da Encefalopatia Espongiforme Bovina, o que deixou a indústria largamente dependente de fontes proteicas de origem vegetal. Atualmente cerca de 40 milhões de toneladas são importadas, representando 80% do consumo de matérias primas proteicas (Hausling 2011).

No caso da soja, a dependência da importação é extremamente elevada, onde a sua produção cobre apenas 5% do seu consumo doméstico. As importações para a EU desta matéria-prima são em média 36.1 milhões de toneladas equivalentes por ano (média entre 2012-2014) (Comissão Europeia, 2016). A EU entende que a forte dependência de importações pode expor o setor a vulnerabilidades na cadeia de suprimento bem como a insustentabilidade da produção de matérias primas em permanecer na sua maioria de origem intercontinental. Assim, torna-se urgente encontrar fontes proteicas alternativas que sejam seguras, acessíveis e sustentáveis para a alimentação animal.

1.2 A produção de insetos e o setor agropecuário

Desde o início da década que a FAO chamou a atenção para a utilização de insetos como fontes nutricionais alternativas para a alimentação humana e animal. Recentemente, com um incremento na investigação e iniciativas para promover os insetos como alternativas nutricionais, os consumidores estão cada vez mais conscientes e revelam um maior interesse nesta área. Todavia, um dos desafios na adoção desta prática prende-se com o facto de que os insetos são muitas vezes descritos com "aversão" e "neofobia", tendo alguns estudos sobre a aceitação dos consumidores revelado uma preferência para o consumo de produtos processados com insetos menos visíveis. Uma forma de promover o seu consumo poderá ser indiretamente através de produtos de origem animal alimentados á base de insetos (Sogari *et al*. 2019).

No setor agropecuário, a escassez de matérias primas para a alimentação animal, particularmente matérias primas proteicas, enaltecendo a insustentabilidade das fontes tradicionais, aumento dos preços e dependência de mercados externos para a sua provisão, levou ao reconhecimento no mercado da proteína de inseto (Vantomme *et al*. 2012). Em adição às propriedades nutricionais dos insetos, a sua produção, dependendo do contexto, requer menos recursos (solo arável e água) comparando com as matérias primas convencionais, pelo que se espera que a sua produção em massa em quantidades semelhantes a farinha de peixe e soja possa reduzir significativamente a pegada ambiental do setor.

O recurso aos insetos no setor agropecuário não se limita apenas como uma fonte de proteína mais sustentável, mas também como uma ferramenta para o maneio de efluentes agropecuários e subprodutos da produção alimentar uma vez que certas espécies possuem a capacidade de converter estes resíduos, reduzindo o seu impacto no ambiente ao mesmo tempo que adicionam valor, contribuindo para um modelo de economia circular.

1.2.1 Propriedades nutricionais dos insetos edíveis

De aproximadamente um bilião de espécies de insetos identificadas, cerca de 2000 estão documentadas como edíveis. Devido á sua vasta diversidade com diferentes habitats, dietas, tipos de metamorfose e estadios de desenvolvimento a sua composição nutricional é altamente variada (Van Huis 2013).

O conteúdo calórico varia entre as 400 e 500 kcal/100g, dependendo da composição, nomeadamente do teor de proteína e gordura. O conteúdo de proteína, varia entre 30% e 60% no teor total de matéria seca. Estes valores são comparáveis com a farinha de soja (~50%) e inferiores á farinha de peixe (~70%). Vários autores identificaram que a ordem dos grilos e gafanhotos possui o conteúdo mais elevado de proteína, com algumas espécies situando-se na ordem dos 70%, assemelhando-se á farinha de peixe. Outras espécies pertencentes às ordens das borboletas, das moscas e dos escaravelhos também apresentam teores elevados de proteína (Rumpold & Schluter 2013; Barroso *et a*l 2014).

Barroso *et al.* (2014) verificou que o perfil de aminoácidos aparenta estar relacionado com a ordem taxonómica, tendo agrupado a ordem das moscas como a mais semelhante á farinha de peixe, em termos de aminoácidos essenciais, e as ordens dos grilos e gafanhotos e dos escaravelhos á farinha de soja.

A seguir a proteína, a gordura é o maior constituinte dos insetos e onde se verifica a maior variabilidade, situando-se entre 13% e 33%. O conteúdo de gordura está relacionado com a espécie, mas também com a sua dieta, idade e estadio de desenvolvimento. Por exemplo, em insetos holometabólicos (metamorfose completa) o conteúdo de gordura é maior nos estadios larvares em comparação com os adultos (Sánchez-Muros *et al.* 2014). Em relação ao perfil de ácidos gordos, Barroso *et al.* (2014) verificou que este se encontra fortemente relacionado com a dieta. De um modo geral, os ácidos gordos insaturados dominam o espetro dos ácidos gordos nos insetos, sendo o rácio saturados: insaturados na maioria das espécies edíveis inferior a 40% (Rumpold & Schluter 2013; Van Huis 2013). Em comparação com a farinha de soja e farinha de peixe, os insetos possuem níveis inferiores de ácidos gordos polinsaturados n3, e maiores quantidades de ácidos gordos polinsaturados n6 do que a farinha de peixe, mas inferiores a farinha de soja (Barroso *et al.* 2014).

Os insetos apresentam conteúdos significativos de fibra, variando entre 5 a 13%, dependendo da espécie e estadio de desenvolvimento. Com base na semelhança estrutural com a celulose, é sugerido que a fibra nos insetos seja maioritariamente composta por quitina. A quitina é um polissacárido que se encontra exclusivamente presente no exosqueleto dos artrópodes, envolvida numa matriz de proteínas, lípidos e minerais (Finke 2007). Vários autores sugerem uma sobrestimação do teor de proteína bruta devido ao teor de nitrogénio (N) que se encontra ligado á quitina.

O conteúdo de cinzas nos insetos varia em média entre 3 e 10%. A maioria dos insetos contem quantidades baixas de cálcio, potássio e sódio, à exceção de algumas espécies de moscas. Adicionalmente, algumas espécies apresentam elevados conteúdos da maioria dos minerais como o cobre, magnésio, fósforo, selénio, ferro e zinco. Os insetos também podem fornecer várias vitaminas, maioritariamente do complexo B como a vitamina B12, riboflavina, acido panteónico e biotina, dependo da espécie (Rumpold & Schluter 2013; Van Huis 2017; Makkar 2018).

1.2.2 Impacto ambiental da produção de insetos

Vários estudos de avaliação do ciclo de vida foram realizados com o objetivo de avaliar o impacto ambiental da produção de insetos. Os estudos de avaliação ao ciclo de vida avaliam parâmetros como o potencial de aquecimento global (medido em CO2 equivalentes) e outros parâmetros ambientais como a utilização de solo arável e energia para quantificar os impactos de um produto ao longo da sua cadeia de suprimento (Halloran 2016).

Em comparação com as espécies pecuárias os insetos foram descritos em possuir uma menor pegada ambiental. Apresentam menores emissões de gases com efeito de estufa, emissões de amónia, e apenas algumas espécies tem a capacidade de produzir metano (Oonincx *et al.* 2010).

Ao contrário dos mamíferos, os insetos são animais ectotérmicos e como tal não despendem energia para regular a temperatura corporal, o que contribui para uma maior eficiência alimentar destes animais. Ainda assim, a eficiência alimentar depende de vários fatores, nomeadamente a espécie e o tipo de dieta consumida. Oonincx *et al.* (2015) reportou diferentes índices de conversão alimentar em quatro espécies de insetos alimentados com a mesma dieta.

Em adição, os insetos apresentam taxas de crescimento rápidas, ciclos de vida curtos e uma elevada capacidade reprodutiva. Outra característica relevante dos insetos, é a sua porção edível, sendo 100% para as larvas e cerca de 80% para os adultos (excluindo pernas e exosqueleto), comparativamente a outras espécies como os frangos e suínos (55%) e bovinos (40%) (Van Huis 2013; Gahukar 2016).

Outra caraterística favorável á sua produção é uma menor necessidade de recursos. Um estudo de avaliação ao ciclo de vida, demonstrou que para produzir 1kg de proteína edível de inseto é necessário apenas 43% da área total utilizada para produzir o equivalente de proteína edível como o leite, e apenas 10% do utilizado para produzir 1kg de carne (Oonincx & De Boer, 2012). Semelhantemente, os insetos requerem menos água para a sua produção, uma vez que são capazes de obter a água de que necessitam diretamente dos alimentos e devido à sua maior eficiência de conversão alimentar (Miglietta *et al* 2015).

Estudos foram conduzidos para comparar a farinhas de inseto para alimentação animal com a farinha de soja e farinha de peixe, revelando um aumento no potencial de aquecimento global. Os autores referiram o incremento na utilização de energia como o principal causador do impacto ambiental da produção de insetos. Este aumento deve-se ao facto de que os insetos necessitam de temperaturas adequadas ao seu desenvolvimento, sendo necessário o aquecimento das instalações quando a temperatura ambiente é baixa. Outros autores referem ainda um aumento da utilização de energia relacionado com o processo de secagem, necessário para posterior inclusão nas rações. Todavia, a produção de insetos a larga escala encontra-se ainda em fase de desenvolvimento tendo potencial para reduzir o consumo energético e as emissões relacionadas. Medidas como a utilização de fontes de energia renováveis, a localização das unidades de fabrico em climas amenos e o uso de espécies com baixos requerimentos de temperatura, podem aumentar a eficiência energética da produção de insetos (Oonincx & De Boer 2012; De Boer *et al*. 2014; van Zanten *et al*. 2015; Salomone *et al.* 2017).

A dieta é um ponto determinante para a sustentabilidade do produto final e para o propósito a que se destina. Nos estudos mencionados, o tipo de dieta consumida pelos insetos foi responsável por grande parte dos parâmetros ambientais avaliados. Por exemplo, em ambos os estudos (Oonincx & De Boer, 2012 e Miglietta *et al* 2015) a mistura de cereais e legumes foi responsável pela maioria da utilização de terra e consumo de água.

Surge assim um grande interesse num número de espécies de insetos decompositores naturais, capazes de converter uma variedade de resíduos orgânicos e subprodutos de baixa qualidade que não são adequados para o consumo humano ou alimentação animal, reduzindo o seu impacto ambiental e aumentando a sua utilidade.

1.2.3 Bioconversão e economia circular

A investigação acerca da utilização de insetos como ferramenta para a valorização de resíduos tem crescido exponencialmente. Este tópico é particularmente relevante dado que anualmente são desperdiçados cerca de 1.3 biliões e 88 biliões de toneladas de alimentos no mundo e na UE, respetivamente (FAO 2014; Stenmarck *et al*. 2016). O desperdício de alimentos ocorre ao longo de toda a cadeia de suprimento, desde a produção agrícola até ao consumidor. Na UE os setores onde ocorre a maioria das perdas (>70%) são ao nível do consumo doméstico e processamento. Em Portugal, estas perdas são estimadas em 1 milhão de toneladas a cada ano, e ocorrem principalmente nas etapas de produção agrícola e maneio pós-colheita e ao nível do consumo, aproximadamente 75% das quais são vegetais, cereais, frutas e lacticínios (Baptista *et al.* 2012).

Este desperdício para além de representar investimentos dissipados em recursos (água, terra, trabalho, fertilizantes, energia) e perdas económicas, causa um grande impacto ambiental através de emissões de CO2, sendo particularmente nocivos quando o seu destino são aterros sanitários dando origem a emissões de metano (FAO, 2014).

Adicionalmente é estimado que 1.4 biliões de toneladas de estrume sejam produzidas na EU a cada ano. Como consequência da intensificação da atividade pecuária, grandes quantidades de estrume são acumuladas em regiões onde há uma grande concentração de explorações, comprometendo a reciclagem dos nutrientes para os solos dando origem a fontes de poluição.

Neste contexto, o recurso aos insetos representa uma oportunidade para adicionar valor aos resíduos orgânicos e diminuir o impacto ambiental associado. Do processo de bioconversão resulta uma biomassa animal que posteriormente poderá ser processada e fracionada em óleo, utilizado como matéria prima para a produção de biodiesel, e concentrado proteico para a alimentação animal. A quitina e os seus derivados são outro potencial subproduto que poderá ser fracionado, com potencial para várias aplicações práticas. O produto residual do processo de bioconversão, consiste em mudas de pele e fezes de inseto, ainda contendo quantidades elevadas de elementos N, P, K e C e outros minerais importantes, podendo ser utilizado como fertilizante orgânico com potencial para substituir os fertilizantes minerais (Newton *et al*. 2005; David Houben *et al* 2020)

Desta forma, os insetos têm uma posição estratégica na cadeia de valor, atuando como uma ferramenta para fechar o ciclo de produção, contribuindo assim para um modelo de economia circular em oposição ao padrão linear de extração, produção, uso e despejo. A reciclagem de subprodutos permite que todos os nutrientes sejam devolvidos á cadeia de valor, tornando os sistemas menos dependentes dos recursos naturais finitos.

Dado que apenas algumas espécies possuem a capacidade de converter determinados tipos de resíduos, a seleção de espécies com características superiores de cultivo é um passo importante para o sucesso do processo de bioconversão. Os insetos pertencentes á ordem Díptera como a *Musca domestica* e a *Hermetia Illucens* estão entre os mais populares devido á capacidade das suas larvas de digerir vários tipos de substratos de origem animal e vegetal. O *Tenebrio molitor* também é uma espécie promissora devido a sua habilidade de consumir matéria orgânica de origem vegetal (Cickova *et al.* 2015; Oonincx *et al.* 2015; Van Huis & Oonicx 2017).

1.3 A mosca soldado negro (*Hermetia illucens*)

1.3.1 Descrição e ciclo de vida

Hermetia Illucens (Linneus,1758), conhecida como a mosca soldado negro (BSF), pertence á ordem Díptera da família *Stratiomydae.* Esta espécie embora originária do continente americano encontra-se atualmente distribuída em zonas tropicais e subtropicais do mundo, tendo o seu primeiro relato em Portugal datado em 1995 (Cickova *et al.* 2015; Martínez-Sánchez *et al.* 2011). A BSF é um inseto holometabólico, pelo que o seu desenvolvimento segue as fases de ovo, larva, pré-pupa, pupa e adulto.

Os adultos são moscas robustas medindo entre 10 a 20 mm de comprimento, corpo preto, duas longas antenas, duas asas e três pares de patas. O aparelho bucal é vestigial, pelo que nesta fase não se alimentam, consumido apenas líquidos para se manterem hidratados (Oliveira & Smith 2016). A fêmea deposita cerca de 700 ovos em uma única postura (Barros *et al* 2019). As larvas resultantes são consumidoras vorazes, ao contrário dos adultos, estas possuem um aparelho bucal funcional e um canal alimentar complexo e enzimas digestivas que as permitem consumir uma ampla variedade de matéria orgânica animal e vegetal (Bruno *et al.* 2020; Kim *et al.* 2011). No seu último estadio larvar, a fase de pré-pupa, exibem um teor elevado de proteína e gordura que as permite desenvolverem-se durante o processo de metamorfose (fase de pupa) e o estadio de mosca.

O ovo inicia o ciclo de vida da BSF e marca o fim do estadio de vida anterior. O ciclo de vida completo tem uma duração de 40 a 45 dias (Tomberlin et al 2002).

1.3.2 Composição nutricional

A literatura disponível revela uma grande variabilidade na composição nutricional das larvas de BSF, relacionada particularmente com a composição do substrato de cultivo, mas também com o estadio de desenvolvimento e métodos de processamento. O teor de proteína bruta varia entre 31-64%, em que os maiores valores parecem estar associados ao seu cultivo em substratos com maior teor proteico e humidade (Oonincx *et al.* 2015; Meneguz *et al.* 2018). O teor de gordura bruta varia entre 21-41%, estando associado com a quantidade hidratos de carbono presentes na dieta (Spranghers *et al.* 2017). O conteúdo de cinzas apresenta a maior variação, entre 2.7-72.2%, e parece estar relacionado com o conteúdo de cinzas do substrato (Spranghers *et al.* 2017). O conteúdo de quitina varia entre 1.4% e 6.7%, sendo que é sugerido por alguns autores que este é influenciado pela duração do desenvolvimento larvar (Diener *et al.* 2009; Meneguz *et al.* 2018)

Os aminoácidos essenciais mais representativos na proteína das larvas BSF são a lisina, leucina e valina. Apesar de existir alguma variação consoante o substrato de cultivo, esta não é tão marcada como nos outros componentes nutricionais (Spranghers *et al*. 2017). Os métodos de processamento também podem interferir com o conteúdo de aminoácidos (Leni & Sforza, 2019). No caso de as larvas de BSF serem desengorduradas para obtenção de óleo e concentrado proteico, os valores de proteína bruta podem alcançar 60%, consequentemente contendo uma composição de aminoácidos superior à da farinha de soja (Spranghers *et al*. 2017).

Em relação ao perfil de ácidos gordos, este é maioritariamente composto por ácidos gordos saturados (entre 61 e 83%). As larvas de BSF são tendencialmente ricas em ácido láurico, chegando a representar até 60% do total de ácidos gordos (Spranghers *et al*. 2017; Oonincx *et al.* 2015).

Vários autores sugeriram que o substrato influencia significativamente no conteúdo de minerais. Ainda assim, o cálcio é o mineral mais abundante nas larvas de BSF, seguido do fósforo, potássio, sódio e magnésio (Newton *et al.* 2005; Tschirner & Simon et al 2015; Spranghers *et al.* 2017; Chia *et al.* 2020).

1.4 Alimentação e nutrição de suínos

Na nutrição de suínos, a adoção de estratégias de alimentação e formulação de dietas que fornecem os nutrientes essenciais em quantidades que vão de encontro às necessidades dos animais são de grande importância, tanto do ponto de vista económico como ambiental. Vários nutrientes são essenciais, no entanto um maior enfase é dado á energia e proteína, sendo estes o os principais constituintes das dietas e responsáveis pela quase totalidade dos custos da alimentação.

A principal fonte de energia nas dietas de suínos são os hidratos de carbono, particularmente o amido, presente em abundância nos grãos de cereais. Os requerimentos energéticos podem também ser supridos com óleos e gorduras, que são fontes densas de energia e ácidos gordos essenciais, aumentam a palatibilidade das dietas e reduzem a poeira e a produção de calor.

Em relação á proteína, maior atenção é dada aos aminoácidos essenciais. O primeiro aminoácido essencial limitante nas dietas para suínos é a lisina seguida pelo triptofano, a treonina e a metionina, pelo que os requerimentos em aminoácidos dos suínos são normalmente expressos em relação á lisina (NRC, 2012).

Dado que a energia e nutrientes contidos nos alimentos não se encontram totalmente disponíveis para os animais, é essencial conhecer o seu verdadeiro valor nutricional. A determinação dos coeficientes de digestibilidade e valor energético são as práticas mais utilizadas para avaliar a biodisponibilidade dos nutrientes nos alimentos e assim determinar o seu valor nutricional.

2. Objetivos

O presente estudo pretendeu avaliar o valor nutricional da farinha não desengordurada de BSF em substituição parcial e completa da farinha e do óleo de soja em dietas para suínos. Para atender ao objetivo foi realizado um ensaio metabólico de modo a analisar a digestibilidade aparente e o balanço metabólico das dietas. Os parâmetros de performance de crescimento também foram avaliados.

3. Materiais e Métodos

Neste estudo foram utilizados 12 suínos machos inteiros de raça cruzada Pietran x (Duroc x Large Whie x Landrace) com um peso vivo inicial médio de 65,52 ± 2,2 kg em fase de acabamento/engorda. Os animais foram divididos em 2 grupos experimentais, com 6 animais cada, de pesos semelhantes (64,75 ± 2,36 e 66,29 ± 1,82 kg). Os animais foram submetidos aleatoriamente a três dietas experimentais, cada grupo com duas réplicas por tratamento (um total de quatro suínos por tratamento). Os animais foram alojados em parques individuais e em caixas metabólicas para a colheita individual de fezes e urina e controlo do alimento ingerido, alternando por períodos de sete dias, num total de 35 dias de ensaio.

As dietas experimentais foram formuladas de modo a conter valores iguais de proteína e energia bruta. A dieta controlo continha farinha de soja com 44% de teor de proteína bruta e óleo de soja (SBM), e para os outros dois tratamentos estes foram substituídos parcialmente (50%) e totalmente (100%) com farinha de BSF não desengordurada (BSF50 e BSF100, respetivamente).

Os animais foram pesados semanalmente para registo do peso vivo e ajuste da quantidade de alimento fornecido (5% do peso vivo). As sobras de alimento eram removidas e contabilizadas para posterior cálculo do alimento ingerido. A colheita e quantificação de fezes e urina eram realizadas diariamente. As amostras de alimento e fezes foram processadas e analisadas para determinação dos parâmetros: matéria seca, proteína bruta, energia bruta, teor de cinzas, fósforo e cálcio. As amostras de urinas foram processadas e analisadas para determinação do nitrogénio total, azoto amoniacal, energia bruta, cálcio e fósforo.

Os pesos vivos semanais foram utilizados para o cálculo do ganho médio diário (ADG). O alimento fornecido e as sobras foram utilizadas para o cálculo da ingestão média diária (ADFI). O ganho de peso total e alimento ingerido total foram utilizados para calcular o índice de conversão (FCR). Estes parâmetros foram calculados para cada período nas caixas metabólicas.

A digestibilidade aparente (ATTD) foi calculada para a matéria seca, nitrogénio total, energia bruta, fósforo e cálcio. O balanço metabólico foi calculado para o nitrogénio total e energia bruta.

Os dados foram analisados estatisticamente com recurso ao Proc Mixed do SAS. Foram testados os efeitos da dieta, incluídos no modelo como fatores fixos. Os resultados foram expressos como a média dos mínimos quadrados e o desvio padrão da média e comparados utilizando o método do teste múltiplo. Os valores de probabilidade inferiores a 0.05 foram considerados estatisticamente significativos.

4. Resultados

Apesar das dietas terem sido formuladas para serem isoenergéticas e isoproteicas houve variações em certos componentes. O teor de proteína bruta foi superior para as dietas com a inclusão de BSF, sendo o maior valor na dieta BSF50 e o menor para a dieta controlo (SBM). O teor de gordura aumentou com a inclusão sucessiva de BSF, assim como o conteúdo calórico. A dieta SBM continha quantidades superiores de cálcio e fósforo comparando com as dietas de BSF. Verificou-se ainda um ligeiro aumento da fibra detergente neutra com a inclusão de BSF nas dietas.

O ganho médio diário (ADG) foi significativamente afetado pela dieta. Quando alimentados com a dieta BSF100 os suínos tiveram um menor ADG comparando com as dietas SBM e BSF50. Os efeitos da dieta não foram significativos na ingestão média diária de alimento e no índice de conversão.

Não houve diferenças significativas entre a digestibilidade aparente (ATTD) das dietas experimentais, exceto para a ATTD da matéria seca e da energia bruta. A ATTD da matéria seca foi significativamente superior na dieta BSF50 em comparação com a SBM. A ATTD da energia bruta foi significativamente superior para as dietas com BSF. O balanço metabólico da energia quando expresso em percentagem de ingestão foi significativamente superior para as dietas de BSF.

Não se verificaram efeitos significativos da dieta na ATTD do cálcio e do fósforo, ainda que tenha havido diferenças significativas na sua ingestão e excreção nas fezes entre as dietas.

5. Discussão

Os estudos do uso de BSF como fonte proteica e energética nas dietas de espécies monogástricas têm vindo a crescer mostrando resultados promissores. Todavia, a informação disponível da sua utilização em dietas para suínos é menos extensa. Os estudos existentes foram maioritariamente realizados em leitões e utilizando baixos níveis de inclusão de BSF (até 10%).

O presente estudo pretendeu avaliar o valor nutricional da farinha de larvas BSF em substituição parcial (50%) e total (100%) da farinha e óleo de soja (níveis de inclusão de 9 e 18%), em que o principal objetivo foi determinar a digestibilidade e balanço dos nutrientes, ainda que os parâmetros de performance de crescimento tenham sido avaliados.

Os resultados deste estudo sugerem que a performance de crescimento de suínos alimentados com dietas contendo farinha de BSF são comparáveis à de suínos alimentados com farinha de soja. Contudo, é importante salientar que os dados de performance recolhidos neste estudo são respetivos apenas aos períodos nas caixas metabólicas, não englobam todo o período experimental e não refletem as condições normais de uma produção de suínos, para além do que, para este propósito, o número de animais (n=12) é reduzido, estando os resultados sujeitos a uma maior variabilidade individual.

A um nível de substituição total da farinha de soja com farinha de BSF (BSF100) os suínos apresentaram uma redução do ganho médio diário em comparação com as dietas SBM e BSF50. Que seja do conhecimento da estudante, existe apenas um estudo que avalia a performance de crescimento em suínos em fase de engorda/acabamento usando níveis elevados de inclusão de farinha de BSF (9, 12, 14.5, 18.5%). Contrariamente ao observado no presente estudo, Chia *et al* (2019) demonstrou que a substituição total de farinha de peixe por farinha de BSF não afetou o ganho médio diário ou o ganho de peso vivo total. Ainda nesse estudo, não foi observado um efeito significativo da dieta no índice de conversão, à semelhança do observado no presente estudo.

A ingestão média diária de alimento foi idêntica entre as dietas experimentais, indicando que as dietas com farinha de BSF são igualmente palatáveis em comparação com a dieta de soja, como já foi anteriormente reportado em outros estudos (Newton *et al* 1997). Num outro estudo em suínos em fase de acabamento, em que foram utilizados níveis inferiores de inclusão de farinha de BSF (4% e 8%), verificou-se que um nível de inclusão de 4% resultou em um aumento significativo do ganho médio diário e do peso vivo final e um índice de conversão menor, em comparação às dietas de farinha de soja e 8% de farinha de BSF (Yu et al. 2019).

As diferenças entre os estudos podem ser explicadas pelos diferentes níveis de inclusão, bem como as propriedades nutricionais da farinha de BSF utilizada nos estudos, que varia substancialmente de acordo com o substrato de cultivo, estadio de desenvolvimento utilizado e os métodos de processamento.

A avaliação da digestibilidade é um meio para estimar a biodisponibilidade dos nutrientes nos alimentos, fornecendo informação valiosa para a determinação dos níveis de inclusão adequados e obter valores precisos para a formulação de dietas. Que seja do conhecimento da estudante, este é o primeiro estudo a avaliar a digestibilidade dos nutrientes em dietas com a inclusão da farinha de larvas BSF em suínos em fase de engorda/acabamento. Contudo, existem alguns estudos realizados em leitões reportando resultados variáveis. No presente trabalho, a inclusão de BSF em substituição parcial resultou numa digestibilidade aparente da matéria seca ligeiramente superior à da dieta controlo. Adicionalmente, a substituição parcial e total da farinha de soja por farinha de BSF resultou numa ligeira melhoria da digestibilidade aparente da energia bruta, não tendo havido diferença entre a digestibilidade do nitrogénio total (proteína) entre as dietas experimentais. Uma vez que as larvas de BSF utilizadas neste estudo continham 27.8% de gordura, o que por sua vez resultou num teor de gordura superior nas dietas de BSF em comparação à dieta controlo, pensa-se que este fator possa ter contribuído para o aumento da digestibilidade aparente da energia bruta das dietas BSF.

Alguns estudos reportaram que a quitina possa ter afetado a digestibilidade da proteína e da gordura, resultando numa performance de crescimento reduzida. As larvas utilizadas neste estudo continham 7.4% de fibra bruta, mas o conteúdo de quitina não foi calculado, pelo que se desconhece até que ponto o teor de quitina possa ter influenciado a digestibilidade dos nutrientes.

Adicionalmente, embora não se tenha verificado uma digestibilidade aparente (ATTD) do cálcio significativa entre as dietas, observou-se uma tendência de aumento da ATTD do cálcio acompanhada de uma menor excreção nas fezes e urina, com a inclusão de BSF na dieta. O mesmo foi observado para o fósforo. Sugerindo que a farinha de BSF pode ser considerada uma fonte de cálcio e fósforo em dietas para suínos.

6. Conclusão

O presente estudo permitiu concluir que a farinha de larvas BSF não desengordurada pode ser considerada como uma fonte adequada de proteína e energia para as dietas de suínos de engorda. A análise dos resultados sugere que esta fonte alternativa demonstra potencial para substituir de modo parcial ou total a farinha e o óleo de soja. Apesar dos parâmetros de performance de crescimento terem revelado alguma desvantagem a uma substituição total, isto não se verificou na digestibilidade dos nutrientes, onde não foram encontradas diferenças discrepantes entre as dietas, e de facto a utilização de farinha de BSF resultou em ligeiras melhorias. De modo a obter dados mais conclusivos nos parâmetros de performance de crescimento é sugerido um estudo com um maior número de animais e em condições semelhantes a uma produção industrial de suínos.

Adicionalmente, é de salientar que a farinha de BSF possui enormes vantagens ambientais, particularmente devido á sua capacidade de aproveitamento de subprodutos locais, com pouco ou nenhum valor para o consumo humano ou animal, reintroduzindo-os de volta á cadeia alimentar como fontes de elevada qualidade nutricional.

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List of Abbreviations

AA – amino acid

ADF -acid detergent fiber

ADFI - Average daily feed intake

ADG - Average daily gain

ADL- acid detergent lignin

AID- apparent ileal digestibility

ASF- animal source food

ATTD- apparent total tract digestibility

BSF – black soldier fly

BWG- body weight gain

Ca- calcium

CH_4 – methane

CO_2 – carbon dioxide

CP- crude protein

DM- dry matter

EU- European union

FA- fatty acid

FAO – Food and Agriculture of the United Nations

FCR - feed conversion ratio

FM- fishmeal

GE- gross energy

GHG- greenhouse gases

INIAV - National Institute for Agrarian and Veterinary Research

LCA- life cycle assessment

N- nitrogen

NDF- neutral detergent fiber

NFE- nitrogen free extract

NH_3 – ammonia

NO_2 – nitrous oxide

P- phosphorus

PUFA's - polyunsaturated fatty acids

SBM- soybean meal

UFA- unsaturated fatty acids

Chapter I – Internship Report

The internship took place at the National Institute for Agrarian and Veterinary Research (INIAV) between 23rd September 2019 and 12th March 2020 as part of a research on the use of insects as feed. The project was also developed at the pilot research unit from a start-up company Ingredient Odyssey SA (Entogreen), specialized on the development of sustainable agri-food solutions, which created a system for valuing agro-industrial waste based on the use of insects as bio converters.

The first part of the internship was spent at the Laboratory of Animal Nutrition where the intern had the opportunity to perform the characterization of animal feed ingredients for general nutritional parameters (dry matter, crude ash, crude protein, crude fat, fiber analysis) following the Portuguese Normatives.

The intern was also at the pilot research unit developing all activities related with the maintenance of the Black Soldier Fly (BSF) colony and the production/processing of the larva for the experimental diets. Beside the intern followed the production of the experimental diets for the trial at the feed experimental factory.

At the pilot research unit, the intern had the opportunity to participate in larva bio digestion trials, including manure bio digestion, and several other by-products. The intern also provided support and participated in project presentations, with EntoGreen, on initiatives promoting insect consumption and project presentations, namely the “World Edible Insect Day” on 26th of October 2019 at the Nova School of Business and Economics, and the project presentation “EntoValor” at INIAV on the 22nd of November 2019.

The metabolic trial lasted from January 22nd to March 4th of 2020. The intern and colleagues were responsible for all tasks related with the research: identification and allocation of the animals, feeding, collecting of samples and productive data, as well as monitoring health. The animals arrived on January 16th, were individually weighed, divided into two experimental groups and allocated into individual pens and metabolic cages. Following a period of seven-day adaptation the metabolic trial started. During the trial, the day began with the collection and measurements of feed refusals, feces and urine, that were stored for posteriorly analysis. The installations were cleaned every day. On Wednesdays the animals were weighed individually, and the groups were switched between the metabolic cages and the pens.

By the end of the trial the pigs were slaughtered by electrical stunning at the experimental slaughterhouse at INIAV. The first group of animals was slaughtered on February 27th and the second group on March 5th. On the days of slaughter, samples and measurements of the organs were performed for investigation on the morphology and histological features, performed and included in Inês Vieira thesis. The carcasses were weight post-slaughter and kept cooled until the next day for posterior butchering. Samples and measurements were performed for carcass traits studies, performed and included in Ana Pestana thesis.

The intern proceeded with the samples related to the metabolic trial which were processed and analyzed at the Laboratory of Animal Nutrition of INIAV.

As mentioned, this research was performed in collaboration with two other students, what contributed to develop new competences in collaborative work and in the management of a long and challenging research process. The results and materials were divided between the three of us and included in which of our thesis, depending on the subject, contributing to a more comprehensive research work.

FIG. 1 Larva and feed production (on top from left to right: egg weighting, larva production, feed manufacture) and sample collection and processing (on top and at bottom from left to right: urine measurements, urine sampling, dried feces samples, milling feces samples)

Chapter II – Introduction to the experimental work

1. Background

Accompanied by a steady increase in the world population, expected to reach 9.1 billion people in 2050, food production will have to increase by some 60% from the current values to be able to satisfy the world's increasing demands (Alexandratos & Bruinsma 2012). The expected increases emerge not only due to demographic expansion, but also to economic growth and migration of populations to urban centers consuming increasingly diversified diets, including an increase in the consumption of animal products. Most of the growth in the consumption of animal source food (ASF) will originate from developing countries where traditionally consumption was very low, but still not reaching the high consumptions practiced in developed countries. For instance, in developed countries per capita consumption of meat is expected to reach from about 28kg in 2007 to 48kg by 2050 and dairy from 52kg to 76kg, whereas in developed nations from 80kg in 2007 to 91kg for meat and for dairy 202kg to 222kg (Herrero *et al.* 2015).

Aggravating to the challenge of increasing food production, natural resources such as land and water are increasingly scarce, polluted and facing competition for other uses (urbanization, industrial uses, biofuels production, etc.). In parallel there is an urgent need to reduce greenhouse gas (GHG) emissions and lessen the effects of climate change, further impacting the balance of natural resources affecting productivity and food availability.

These challenges require changes in the production paradigm, towards a more efficient use of available resources and inputs while reducing the impact on the environment, also referred as the sustainable intensification (Herrero *et al*. 2015). However, sustainability will also be required on the demand side. Many studies suggest that changes in consumption patterns could help reduce food-related impact on environment and GHG emissions. For instance, practicing modest ASF consumption and reduce food waste and loss could play a significant role for sustainably feed the growing world population (Van Huis 2013; Schader *et al*. 2015; Herrero *et al*. 2016).

Food production, and particularly the production of animal products, is definitely a major user of natural resources and has a great impact on the environment. For the livestock sector it uses 75% of all agricultural land, contributes to climate change for about 14.5% of the global anthropogenic greenhouse gas (GHG) emissions, and is responsible for almost two-thirds of the anthropogenic ammonia emissions contributing significantly to acidification of soils and eutrophication of surface waters (Steinfeld *et al.* 2006**;** Gerber *et al*. 2013; Herrero *et al*. 2015). Thereby, the livestock sector also plays an important role in adopting more efficient measures in the use of natural resources while safeguarding the environment and reducing its emissions, contributing to the sustainability of food production.

The availability of affordable and quality feed and its feeding is the basis of animal production systems. Financially it is the most important element, representing 70% of the total production costs (Makkar 2016). On the other hand, it is particularly resource demanding and responsible for a large part of the environmental impact of livestock production. Currently, the area used for the production of animal feed reaches about 560 million hectares or 40% of the global arable area, using a significant part of the nearly 200 million tons of fertilizers applied annually and about 8% of water for human use (Mottet *et al.* 2017; FAO 2018). The production of feed also plays a significant role in the emission of GHG, which accounts for 45% of the sector's emissions (Gerber *et al*. 2013). These emissions are related to the manufacture of fertilizers and pesticides for feed production, feed production itself, manure deposition and application, and emissions related to land-use change.

Moreover, the intensification of livestock production leading to increasing amounts of manure accumulated in some regions is a further concern. While manure can be a valuable fertilizer it can also lead to environmental pollution and GHG emissions when not properly handled and applied. The nutrients contained in feeds are often provided in excess to the animal's requirements, or these are poorly available and not efficiently digested being eliminated in feces and urine. For instance, between 55 to 95% of nitrogen and about 70% of phosphorus ingested by livestock is excreted (FAO 2018). These nutrients present in livestock effluents under certain conditions of storage and processing leads to emissions of methane (CH_4) and nitrous oxide (NO_2). The decomposition of organic matter by methane and carbon dioxide- producing bacteria leads to CH_4 emissions, while NO_2 is produced through processes of nitrification and denitrification of the nitrogen contained in manure, mostly present in its organic (proteins) and inorganic form as ammonia (NH3). Furthermore, as a consequence of unbalanced application in the fields, nitrogen and phosphorus losses can reach ground and surface waters as a result of run off or leaching processes, causing eutrophication and ecological deterioration of ecosystems (Gerber *et al*. 2013; Herrero *et al*. 2016; Tullo *et al*. 2019).

In the swine production chain, feed production accounts for the majority of the GHG emissions (60%) mostly related with the land-use change due to the increasing demand for feed ingredients, being soybean a major driver, and the storage and processing of manure being the second larger contributor of pig production related GHG emissions (MacLeod et al. 2013). Hence, two particularly important challenges within pig production are the development of more sustainable diets and effective manure management strategies.

1.1 Feed demand landscape

The global production of compound feed grows every year, following the increase in consumption of ASF. Globally, total compound feed production was estimated at 1.1 billion tones in 2018, representing a 3 % increase from the 2017 estimate (www.allaboutfeed.net). Approximately 44% of the total feed was produced for poultry, followed by around 26% for swine and 22% for ruminant.

Compound feeds are manufactured from a mixture of raw materials designed to achieve pre-determined performance objectives depending on the species and age of the animal. Currently the main ingredients used in feed formulation include a variety of cereal grains and protein supplements such as fishmeal and soybean meal, but also agricultural by-products such as dried distillers' grains, pulps and molasses.

An increasing concern to global food security is the use of human-edible ingredients as feed instead of direct consumption by humans. This is because food provision via animals entails large conversion losses, and also competes for a limited global area of arable land to produce grain crops. For instance, in 2012-2013 approximately 800 million tones, representing one third of all cereal harvest was used as feed and estimates suggest that this share could reach half of all cereal production by 2050 to meet the increasing demand for animal protein products (Makkar 2018).

The projected demand for cereals is the result of several interconnected trends. One of the major reasons is the transition from land-based extensive ruminant systems to large-scale production systems, particularly of monogastric species. Despite the greater efficiency of poultry and pig comparing to ruminants (typically 2 to 4kg of grain vs 7kg for kg of meat produced), these species have a reduced capacity to digest complex molecules such as grass fibers, and therefore require greater amounts of grains and protein edible by humans (Mottet *et al*. 2017). In fact, in industrialized countries intensive production systems are the source of a large part of the global poultry and swine production, and are growing exponentially in developing countries, mainly China and India, to respond the quick demand for ASF (Steinfeld *et al*. 2006; Thornton 2010; Cao & Li 2013; Herrero *et al*. 2015).

Another dimension of the competition for grains is the growing industry of first-generation biofuels (bioethanol and biodiesel) which are primarily made from food crops. Cereals such as corn, wheat, sugar beet and sugar cane are used for bioethanol production and vegetable oils (rapeseed, soya beans and palm oil) for biodiesel. The use of certain crops for biofuels production also results in co-products such as dried distiller's grains that can be introduced into compound feed, however the presence of anti-nutritional factors or poor availability limits its inclusion in feed rations (Cooper & Weber 2012; Popp *et al*. 2016; Salter *et al.* 2017).

The increased demand for swine and poultry meat - expected to grow 38% and 104%, respectively- will imply a greater demand for feed protein (IFIF online). The main protein sources include oilseed meals, processed animal proteins and by-products. Soybean meal (SBM) is the premier protein source in diets fed to pigs and poultry because of its high protein content (up to 50%), better amino acid profile and high digestibility comparing to the plant alternatives (de Visser *et al.* 2014). From the global output, more than 80% of the world's soybeans are processed into soybean meal and oil, with the meal almost entirely used as feed, mostly for pig and poultry production (respectively 29% and 53%), but also in aquaculture (8%) and dairy farming (2%) (Fraanje & Garnett 2020).

However, sustainability of soybean production and distribution has been a subject of increasing concern. The widespread consumption of soybeans is supplied by a few large exporting countries concentrated on the American continent, mainly the United States, Brazil and Argentina. In 2018/19 these three countries together were responsible for 82% of the global soy production (Fraanje & Garnett 2020). Moreover, the land used for soya production by just these three main producers is about 90 million hectares. Nonetheless, while productivity per hectare of crops has increased, largely due to intensification of production, expansion remains an important concern, particularly if involves the destruction of forests and habitat loss.

On the other hand, fishmeal (FM) and fish oil are considered the most nutritious and digestible ingredients in diet formulation for farmed fish, and also for the pig and poultry sector for younger animals. However, FM production depends on the availability of wild harvested marine fish stocks which most are currently fully exploited or overexploited. This have resulted in a decrease in fishmeal availability and increasing prices undermining the profitability of many enterprises in the fast-growing aquaculture production. Whereas the inclusion rates of FM have been showing a downward trend this was compensated by an increasing use of soy, oils and cereals, although these have low protein digestibility and less favorable amino acid patterns (Stamer 2015; Dicke 2018; Zarantoniello et al. 2018).

In the European Union (EU) the feed sector faces a long-term challenge of shortage of protein meal. The ban of meat and bone meal as feed after the Bovine Spongiform Encephalopathy crisis in 2001 is one of the reasons for the current situation, which left the industry almost dependent on vegetable protein sources (Brookes 2001). Currently imports are over 40 million tons, representing 80% of the consumed plant protein rich feed materials (Hausling 2011). The import dependency is extremely high in the case for soy, where EU production covers only 5% of its domestic consumption. Soybean imports to the EU are on average 36.1 million tons of soybean equivalent per year (average 2012-2014) (European Commission 2016).

The EU understands that the strong dependence on imports can expose the sector to vulnerabilities in the supply chain as well as the unsustainability of the production of raw materials in remaining of intercontinental origin. Thus, there is an urgent need to find alternative protein sources that are safe, accessible and sustainable for feed.

2. Insect production and the livestock industry

In the present context of concern to global food insecurity, climate change and global population expansion, the Food and Agriculture of the United Nations (FAO) has drawn attention to the use of insects as alternative nutritional sources either as human food or animal feed. Recently, with a growing body of research and initiatives to promote insects as nutritional alternatives, consumers are increasingly aware and show greater interest in this area.

However, some of the challenges of adopting entomophagy rely on the fact that insects are often described with “disgust” and “neophobia”, with some studies on consumers acceptance revealing that more processed and less visible insects tend to be preferred. Thus, a way to promote entomophagy might be indirectly through insect-fed animals (Sogari *et al*. 2019).

In the livestock industry the growing scarcity of feed ingredients, particularly protein-rich materials, highlighting the unsustainability of the traditional sources, increasing prices and dependence on external markets for provision, has led to market recognition of insect protein (Vantomme *et al*. 2012). In addition to the nutritional properties of insects, insect production depending on context requires fewer resources (water and land) as compared to conventional feed materials. Therefore, is expected that the mass production of insects for feed in quantities comparable to the common feed ingredients could significantly reduce the environmental footprint of livestock production.

Insects can further contribute to increasing the sustainability of animal production systems by offering tools for waste management, since a number of insect species have the ability to convert organic side streams including animal waste (manure and other agricultural waste) reducing environmental contamination while at the same time generating valuable products, thus contributing to a circular economy model.

2.1 Nutritional properties of edible insects

Within the approximately one billion insect species identified, there are some 2000 edible species documented, most of which are native from the tropical and subtropical regions of the world, where they are harvested from the wild and consumed by the local populations (Van Huis *et al*. 2013; Doberman *et al.* 2017). The great diversity of species, habitats and diets, as different types of metamorphosis and development phases, makes the nutritional composition of edible insects highly variable (Van Huis 2013) (Table 1.).

Table 1. Nutritional composition of insects divided by orders with some examples and average values for each order. Mean values of nutrient contents of all insects belonging to the same insect order are indicated in bold. Data from Rumpold and Schluter (2013).

	Protein (% dry matter)	Fat (% dry matter)	Fiber (% dry matter)	NFE (% dry matter)	Ash (% dry matter)	Energy (Kcal/100g)
Blattodea (cockroaches)	**57.30**	**29.90**	**5.31**	**4.53**	**2.94**	
Coleoptera (beetles, grubs)	**40.69**	**33.40**	**10.74**	**13.20**	**5.07**	**490.30**
Rhynchophorus phoenicis (palm weevil larva)	41.69	37.12	-	-	3.27	478.60
Tenebrio molitor (mealworm larva)	47.18	43.08	7.44	0.26	3.08	577.44
Tenebrio molitor (adult)	60.20	20.80	16.30	0.01	2.70	427.90
Diptera (flies)	**49.48**	**22.75**	**13.56**	**6.01**	**10.31**	**409.78**
Musca domestica (larve)	63.99	24.31	-	1.25	5.16	552.40
Eristalis sp.	40.68	11.89	13.27	8.21	25.95	-
Hemiptera (true bugs)	**48.33**	**30.26**	**12.40**	**6.08**	**5.03**	**478.99**
Hymenoptera (ants, bees)	**46.47**	**25.09**	**5.71**	**20.25**	**3.51**	**484.45**
Isoptera (termites)	**35.34**	**32.74**	**5.06**	**22.84**	**5.88**	
Lepidoptera (butterflies, moths)	**45.38**	**27.66**	**6.60**	**18.76**	**4.51**	**508.89**
Bombyx mori (silkworm larva)	53.76	8.09	6.36	24.43	6.36	389.6
Odonata (dragonflies, damselflies)	**55.23**	**19.83**	**11.79**	**4.63**	**8.53**	**431.33**
Orthoptera (crickets, grasshoppers, locusts)	**61.32**	**13.41**	**9.55**	**12.98**	**3.85**	**426.25**
Acheta domesticus (house cricket adult)	66.56	22.08	22.08	2.60	3.57	455.19
Acheta domesticus (nymphs)	70.56	17.74	14.92	-	4.84	-
Melanoplus mexicanus	77.13	4.22	12.17	4.04	2.44	-

The average energy content varies between 400 to 500 Kcal /100g, and will depend on insect composition, namely the protein content and especially the fat content. The main constituent of insects is protein, comprising 30 to 60 % of the total dry matter basis (table 1). These values are comparable to soybean meal (~50%) and lower than fishmeal (70%). In general, the order Orthoptera (crickets, grasshoppers, locusts) has the highest crude protein (CP) contents with some insect species reaching values in the range of 70% CP resembling fishmeal or even higher. The highest contents of CP can also be observed for several species of Lepidoptera (butterflies, moths), Diptera (flies) and Coleoptera (beetles, grubs) (Rumpold & Schluter 2013; Barroso *et a*l 2014).

The amino acid composition of insects seems to be related to insect taxonomy. The order Diptera appears to be the most similar to fishmeal in terms of essential and limiting amino acids, especially *Hermetia Illucens, Musca Domestica* and *Eristallis sp.*, whereas the Orthoptera and particularly Coleoptera orders resemble soybean meal (Barroso *et al* 2014).

After protein, fat is the second largest constituent of insects and the most variable, ranging from 13 to 33%. This variability is not only due to species, but also diet, age and stage of metamorphosis. For instance, in holometabolous insects (complete metamorphosis) the fat content is higher in larval stages than in adult (Sánchez-Muros *et al.* 2014). The larva of some insect species and adults with soft body like termites and palm weevil larva have the highest levels of fat (33% and 37%, respectively), and insects with hard exoesqueleton like crickets and grasshoppers have the lowest contents (13%) (Van Huis 2013; Dobermann *et al*. 2017).

The fatty acid (FA) profile is more dependent on diet in contrast with amino acid profile which is more related to taxon (Barroso *et al.* 2014). In general, unsaturated fatty acids (UFA) dominate the spectrum of FA’s in insects, with the ratio saturated: unsaturated fatty acids in the majority of edible insect species being less than 40%. In relation to soybean and fishmeal, insects possess lower levels of polyunsaturated fatty acids (PUFA’s) n3, and higher PUFA’s n-6 than fishmeal but lower than soybean meal (Barroso *et al.* 2014).

Insects also contain significant amounts of fiber with average contents varying between 5 to 13%, depending on species and development stage (Sánchez-Muros *et al* 2014). Fiber in insects can be measured in crude fiber, neutral detergent fiber and acid detergent fiber (Finke 2007). Based on the structural similarity to cellulose, it is suggested that chitin is the most common form of fiber in insects. Chitin it’s a polysaccharide present exclusively in the exoesqueleton of arthropods, and for insects it is found on the cuticle (an extracellular layer that covers the complete external surface) involved in a matrix with proteins, lipids and other compounds such as minerals (Finke 2007). Chitin is bounded to amino acids from proteins in a cross-linked connection (sclerotization) responsible for the stiffness of the insect’s cuticle (Van Huis 2017). This can interfere with the estimation of crude protein due to unavailable N that is bounded to chitin (Barroso *et al*. 2014). Furthermore, the NFE (nitrogen free extract) content representing carbohydrates other than fiber range between 5 to 23% (Rumpold & Schluter 2013).

The average ash contents of edible insects vary between 3-10%, with the lowest values for Blattodea (cockroaches) and the highest for Diptera orders. Insects are good sources of minerals, yet levels are variable depending on species and diets (Doberman *et al*. 2017). The majority of insects contain small amounts of calcium, potassium and sodium, with exception of *Hermetia Illucens* and *Musca Domestica* (Rumpold & Schluter 2013; Makkar 2018). A number of species such as crickets and termites are high in most minerals like copper, magnesium, phosphorus, selenium, iron and zinc (Rumpold & Schluter 2013; Van Huis 2017).

Insects also provide with several vitamins, mainly from B complex (Van Huis 2017). Vitamin B12 is found in abundance in *Tenebrio mollitor* and the house cricket, in contrast to other species that only contain negligible amounts (Rumpold & Schluter 2013). Insects are normally rich in riboflavin, pantothenic acid and biotin. The insects from Orthoptera and Coleoptera orders can also be good sources of folic acid (Rumpold & Schluter 2013). Conversely, insects are not an efficient source of vitamin A, C, niacin, although some species from Lepidoptera and Termites were found to have significant amounts of retinol and B-carotene (Rumpold & Schluter 2013).

2.2 Environmental impact of insect production

Several life cycle assessment (LCA) studies have been carried out with the objective of assessing the environmental impact of insect production. Life cycle access studies assess parameters such as global warming potential (measured in CO2- equivalents) and other environmental parameters such as the use of arable land and energy to quantify the impacts of a product along its supply chain (Halloran 2016).

In comparison with the conventional livestock species, insects have been found as having a lower ecological footprint. They were found to produce much lower greenhouse gas emissions, ammonia emissions, and only a few species (representatives of cockroaches, termites and scarab beetles) have the capacity to produce methane (Oonincx *et al.* 2010). In the same study Oonincx *et al* (2010) found the production of CO2 to be equal or lower, which is associated with a high feed conversion efficiency.

Unlike mammals, insects are ectothermic animals and as such do not expend energy to regulate body temperature, which contributes to a greater feeding efficiency for these animals. Nonetheless, feed conversion efficiency is dependent on a variety of factors such as species and type of diet consumed. For instance, Oonincx *et al.* (2015) reported different feed conversion rates in four insect species fed the same diet.

In addition, insects have fast growth rates, short life cycles and a high reproductive capacity. Another relevant characteristic of insects is their edible portion, which is 100% for larva and about 80% for adults (excluding legs and exoskeleton), compared to other species such as chickens and pigs (55%) and cattle (40%) (Van Huis 2013; Gahukar 2016).

Furthermore, another characteristic favorable to its production is a lesser need for resources. An LCA study conducted by Oonincx & De Boer (2012), demonstrated that to produce 1 kg of edible protein of insect it is necessary only 43% of the total area used to produce the equivalent of edible protein such as milk, and only 10% of the used to produce 1 kg of meat. In animal production the water needed to grow the food represents the main factor and therefore the water footprint of animal products depends on three factors: quantity, diet composition, and food origin. Insects require less water for their production since they are able to obtain the water they need directly from food and due to their greater efficiency of feed conversion (Miglietta *et al* 2015). However, energy use in insect production was found out to be higher than poultry and dairy production, similar to swine and lower that cow meat production.

Research was also carried comparing insect-based feed with the benchmark's soybean meal and fishmeal, finding an increase in the global warming potential and energy use but a decrease in land use (van Zanten *et al*. 2015; Salomone *et al.* 2017). The authors mentioned the increase in energy use as the main contributor of the environmental impact of insect production. This increase is due to the fact that insects need adequate temperatures for their development, requiring the heating of the facilities when the ambient temperature is low. Other authors also report an increase in the use of energy related to the drying process, required for later inclusion in compound feed (De Boer *et al*. 2014; Salomone *et al.* 2017). However, large-scale insect production is still under development which offers potential to reduce energy consumption and related emissions. Measures such as the use of renewable energy sources, the location of production facilities in warmer climates and the use of species with low temperature requirements during rearing, can increase the energy efficiency of insect production (Oonincx & De Boer 2012;_De Boer *et al*. 2014; van Zanten *et al*. 2015; Salomone *et al.* 2017). Nevertheless, insect production offers significant benefits in terms of land use, as the availability of arable land is the most stringent limitation to sustainably feed the growing world population, as well as slowing down the expansion of agricultural land, which is the main source of GHG production (Foley *et al*. 2011).

Furthermore, the insect diet is a determining point for the sustainability of the final product and for the purpose for which it is intended. In the studies mentioned, the type of diet consumed by insects was responsible for a large part of the environmental parameters evaluated. For example, in both studies (Oonincx & De Boer, 2012 and Miglietta *et al* 2015) the mixture of cereals and vegetables was responsible for the majority of land use and water consumption.

If insects depend on the same resources, such as grains and other food materials, they will compete for the same resources as other production animals and humans, and therefore can be subjected to the environmental and production costs related to feed (Halloran *et al*. 2016). Furthermore, the use of insects fed with quality diets becomes unacceptable for feed. Thus, there is a great interest in a number of species of natural decomposing insects, capable of converting a variety of organic residues and low-quality by-products that are not suitable for human consumption or animal feed, reducing their environmental impact and increasing their usefulness.

2.3 Bioconversion and the circular economy

Research on insect-based bioconversion as a remarkable solution for waste valorization has been growing exponentially. This topic is particularly relevant given that around 1.3 billion and 88 billion tons of food are wasted each year in the world and in the EU, respectively (FAO 2014; Stenmarck *et al*. 2016). Food wastes and losses occur along the entire supply chain from agricultural production to the consumer. In the EU, the sectors where most losses occur (> 70%) are at the level of domestic consumption and processing. In Portugal, these losses are estimated at 1 million tons each year and occur mainly at the initial (agricultural production and post-harvest handling) and final stages (consumption) of the food supply chain, approximately 75% of which are vegetables, cereals, fruits and dairy products (Baptista *et al*. 2012).

This waste, in addition to representing dissipated investment in resources (water, land, labor, fertilizers and energy) leads to loss of economic value, unnecessary CO2 emissions, and is particularly environmental damaging when thrown into landfills and is converted into methane, which has a global warming potential 25 times higher than CO2 (FAO, 2014).

In addition, 1.4 billion tons of manure are produced in the EU each year (European Commission, 2014). As a consequence of intensification of livestock production, confined livestock operations are often concentrated in some regions where significant amounts of manure are generated outstripping the soils capacity and thus becoming a source of pollution.

In this context, the use of insects represents an opportunity to add value to organic waste and reduce the associated environmental impact. The bioconversion process results in an animal biomass that can be processed and fractionated into oil, used as raw material for the production of biodiesel and protein concentrate for feed (Newton *et al*. 2005; Surendrá *et al*. 2016). Furthermore, contributing to decrease the competition between land used for cereals for human consumption, animal feed and biodiesel production (Salomone *et* al. 2017).

Chitin and its derivatives are another potential by-product that can be fractionated, with the potential for several practical applications, namely for the textiles, biomedical, pharmaceutical, paper and cosmetics industries (Ravi *et al*. 2020)

The residual product that results from the process, consisting in molting skins (exuviae) and insect feces ("frass"), still containing high amounts of key elements (nitrogen, phosphorus, potassium, carbon) and other important minerals can be used as an organic fertilizer, showing potential to replace partially or completely mineral fertilizers (Newton *et al*. 2005; Houben *et al.* 2020).

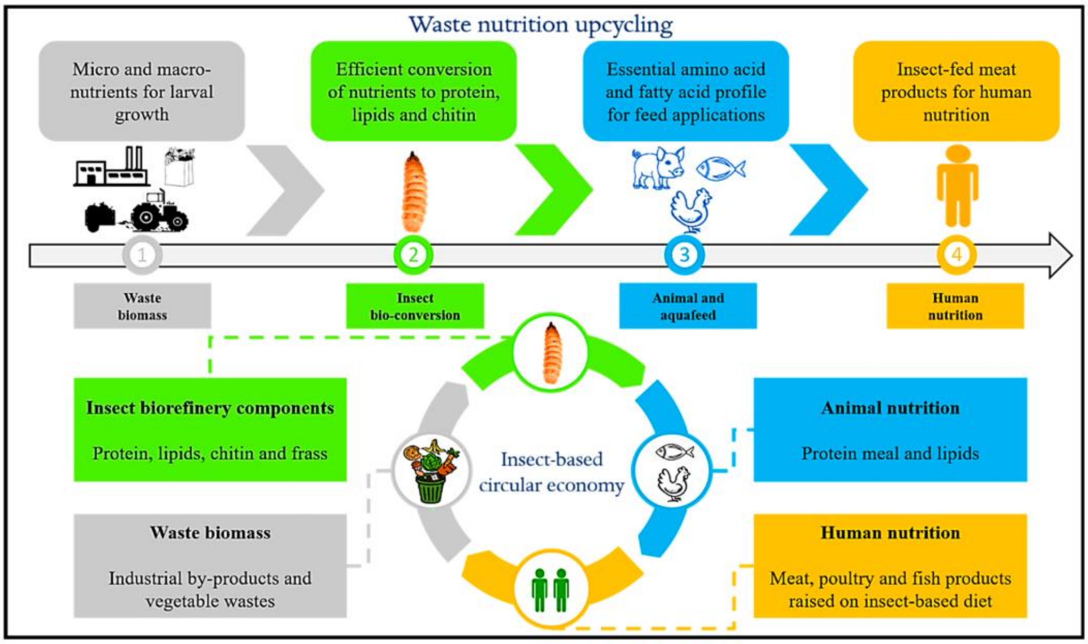

FIG.2 Nutrition upcycling from waste streams and circular economy: from Ravi *et al*. 2020.

In this way, insects have a strategic positioning in the food value chain acting as a tool to close the production cycle and thus contributing to a circular economy model overcoming the linear model of “extraction, production, use and disposal”. The upcycle of by-products allows the return of all nutrients back to the food value chain making systems less dependent on finite resources.

Given that not all insect species have the capacity to convert most types of waste, the selection of species with superior cultivation characteristics is an important part of the success of the bioconversion process. Insects belonging to the Diptera order such as the common house fly (*Musca domestica)* and the black soldier fly (*Hermetia Illucens)* are among the most popular due to the ability of their larva to digest various types of substrates of animal and plant origin. The yellow mealworm (*Tenebrio molitor)* is also a promising species due to its ability to consume plant material (Cickova *et al.* 2015; Oonincx *et al.* 2015; Van Huis & Oonicx 2017).

3. Black soldier fly

3.1 Description and life cycle

Hermetia Illucens (Linnaeus,1758) is a true fly or two-winged fly (Diptera) of the Sratiomyidae family, commonly known as the black soldier fly (BSF). Originally endemic to the American continent, transportation of food and other materials by man enabled its establishment throughout tropical and temperate regions across the globe, with the first record of BSF appearance in Portugal in 1995 (Cicková *et al.* 2015; Martínez-Sánchez *et al.* 2011). The BSF is a holometabolous insect, as so it develops through a complete metamorphosis life cycle including egg, larval, pre-pupal, pupal and adult stages (figure 3).

FIG.3 BSF life stages (from the left to right and from upper to down) 1- adult resting 2- copulation 3- female oviposition 4- eggs 5- larva feeding 6- prepupa. (Author and colleagues original)

Adults are robust flies ranging from 10 to 20 mm in length with a predominant black body with blue or green metallic reflections on the thorax, two elongated antennae and three pairs of feet with white tarsi (Diclaro & Kaufman 2009**;** Oliveira & Smith 2016). There is no obvious external sexual dimorphism, but the female is usually larger than the male. Genital structure represents the sexual dimorphism of this species and can be observed in the last abdominal segment, ending in a plate-like structure for males, and in a scissor shaped structure for females (Chia 2019**;** Oliveira & Smith 2016). The adult BSF possess a sponge-like mouth part that allows them to lap up liquids to stay hydrated, essential for reproduction and correlated with longevity (Oliveira & Smith 2016; Tomberlin et al 2002). The mandibles are absent and other structures reduced which makes it a nonbiting fly (Oliveira & Smith 2016).

Adults are not strong fliers, in nature they spend most time of the day resting on vegetation, and not tend to approach humans or animals. Adult males aggregate in resting areas, competing and defending the area from other males, while waiting for females ready to copulate. This unique lekking behavior is critical for mating (Tomberlin & Sheppard 2001). Matting occurs during the flight and copulation occurs while they are descending to the ground or at the ground. Moreover, mating behavior is significantly correlated with time of the day and light intensity (Tomberlin & Sheppard 2002).

After copulation the female enters in oviposition for a period of two days, where it searches for suitable breeding sites. The eggs are laid in protected cavities and near decomposing materials that will provide a food source for the newborn larva (Dortmans *et al.* 2017). The female BSF produces a single clutch that contains between 620 and 700 eggs. The eggs are elliptical, elongated, rice-shaped, measuring 1-1.4 mm in length and 0.4-0.6 mm diameter, with a cream white color in the first hours and turning to yellowish as the embryo matures (Barros *et al* 2019).

The newly hatched larva are polyphagous and voracious consumers. In contrast with the adult flies, larva possess functional mouthparts, a complex alimentary canal and digestive enzymes that allows them to feed, grow and develop in a wide range of decaying plant and animal matter (e.g.: manure, food scrapes, municipal garbage, rotting plant material) (Bruno *et al.* 2020; Kim *et al.* 2011; Cicková *et al.* 2015). The larva develop trough six instars (period between two molting events that involve renewing of the exoskeleton), where they grow from a few millimeters size to around 2.5 cm length and 0.5 cm width (Chia, 2019).

The sixth instar culminates with prepupal stage. In this stage the exoesqueleton darkens and the larva replaces its mouthpart with a hook shape structure that it uses to move out from the food source in search of a dry environment. At this stage, the prepupae have evacuated their digestive tract and are at their maximum size, exhibiting large protein and fat contents to sustain them through metamorphosis (Newton *et al* 2005; Diener *et al* 2011). When the pupa finds a suitable location, it becomes immobile and stiff and the pupation initiates (Dortmans *et al.* 2017).

Development time of BSF depends on abiotic (e.g., temperature, relative humidity, photo phase) and biotic (e.g., diet, density, and strain) factors. Abiotic conditions are particularly important for insects because of their small size and the proportional large surface which increases water loss (Chia 2019). Considering that BSF is an equatorial and generally a warm temperate season specie it flourishes at warmer temperatures, with almost all oviposition occurring at >26ºC (Tomberlin & Sheppard 2002). In Portugal, the BSF activity is from April to November, peaking in August (Martínez-Sánchez *et al.* 2011).

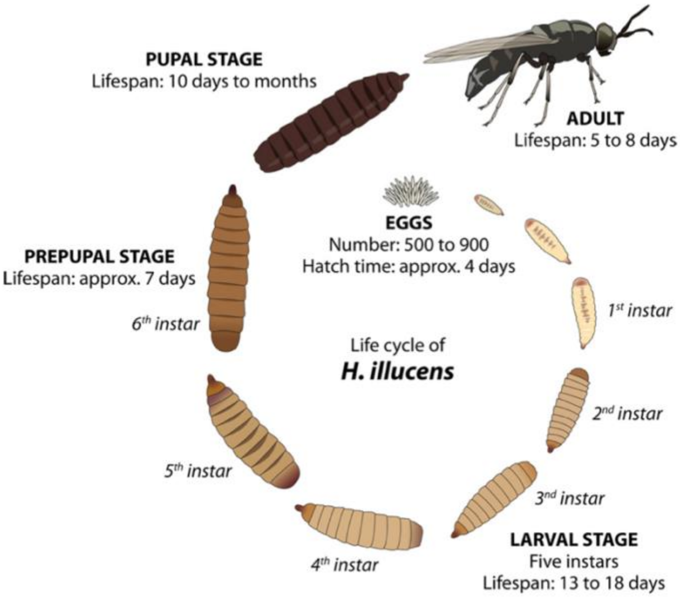

FIG.4 Life cycle of *Hermetia Illucens* (De Smet *et al.* 2018)

The egg starts the BSF life cycle and at the same time ends the previous life stage (fig. 4). The complete lifecycle takes about 40 to 45 days (Tomberlin et al 2002). Eggs hatch into larva in about four days at temperature >26ºC and humidity >60% (Tomberlin & Sheppard 2002). Under ideal conditions with abundant food sources the larva will develop into prepupa in a period of 14-16 days. However, the BSF is a very resilient organism and can resist to demanding environmental conditions such as drought, food shortage or oxygen deficiency, possibly extending the larval period up to four months (Diener *et al.* 2011). Prepupae migrate from the larval habitat to pupate and adults emerge 2 weeks later but it can take up 2 to 5 months depending on environmental conditions (Tomberlin & Sheppard 2002).

3.2 Benefits

The BSF have several characteristics that makes it a suitable specie for mass scale production: short life cycle, high oviposition rate, high survival, high conversion rate, ability to live in high densities. It also has several advantages over other insect species particularly due to their behavior: the adult does not feed or bite due to the lack of functional mouthparts and also it is not attracted to human habitats and food, thus reducing the risks to carry and spread diseases, conversely to the common housefly (Diener *et al* 2011; Makkar *et al* 2014). Moreover, the prepupa stage of the BSF may offer other advantages: the emptying of the gastrointestinal tract which reduces the risk of carrying potential pathogens, and the self-harvest behavior that can be used in mass production systems (Spranghers *et al.* 2017).

Nonetheless, the BSF larva ability to convert a wide spectrum of organic materials might be the biggest advantage over many other insect species, which can be a valuable tool to solve many environmental problems. For instance, it has been demonstrated to convert various types of manure (dairy, swine and poultry) not only reducing volume (50-60%) but also several nutrient contents (mainly N 40-80% and P 60-80%) in short periods of time (Sheppard et al. 1983; Newton et al. 2005; Myers et al. 2008). BSF also contains antimicrobial peptides and have been found to reduce populations of foodborne pathogens in manures (Eirkson 2004; Liu et al. 2008; Elhag et al. 2017). In addition, BSF larva have also proven to efficiently convert several agricultural by-products and food waste such as dried brewer's spent grains, brewer's yeast, cane molasses, vegetable and fruit waste, fish offal, beet molasses, potato steam peelings, bread and cookie remains (Oonicx *et al.* 2015; Chia *et al.* 2018; Spranghers *et al.* 2017; Meneguz *et al*. 2018).

Thereby, the opportunity arises to transform a variety of organic side streams that otherwise would be wasted into animal biomass rich in fat and protein with valuable uses such as animal feed and biodiesel production. The BSF biomass can be fractionated through mechanical (mechanical pressing) or chemical (solvent extraction) means originating high protein meals that can be used both as protein and energy source in animal diets (Tschirner & Simon et al 2015; Surendrá *et al.* 2016). The removal of some of the fat can be beneficial for feed purposes, because it not only improves protein content but is also important for an efficient mixing of feed ingredients and improve storage.

The BSF fat can be further converted into biodiesel via transesterification. Li *et al*. (2011) reported BSF derived biodiesel to contain certain properties such as density, viscosity to name a few, comparable to biodiesel produced from other crops such as rapeseed oil, and to be within the European biodiesel standard EN14214.

However, since insects that are reared within the European Union are considered to be "farmed animals" these can only be produced with substrates eligible as feed materials, whereby the use of substrates such as manure and food waste containing products of animal origin it is prohibited. Nonetheless, vegetable waste and crop by-products less suitable for direct livestock consumption can represent good substrates for insect rearing. Further on, the safety of BSF biomass reared on bio wastes should be assured.

3.3 Nutritional composition

The available literature has highlighted a great variability in the BSF larva nutritional composition, mainly due to diet composition but also development stage and processing methods. The variation in BSF larva composition reared on different diets is reported in table 2.

Table 2. Chemical composition of BSF larva reared on different substrates

Feed substrate	Stage at harvest	DM %	CP % (N-to-P conversion factor of 6.25)	EE%	Chitin %	Ash %	Gross energy (kcal/100g)	References
Poultry manure	prepupa	-	42.1	34.8	-	14.6	-	Newton *et al.* 2005
Swine manure	prepupa	-	43.2	28	-	16.6	-	Newton *et al.* 2005
Liver	prepupa	44.7	62.7	25.1	-	-	214	Nguyen *et al*. 2015
Fish	prepupa	46.6	57.9	34.6	-	-	233	Nguyen *et al*. 2015
Food manufacturing by-product mixes	prepupa	33-36	38-46	21-35	-	-	-	Oonincx *et al.* 2015
Chicken feed	prepupa	38.7	41.2	33.6	6,2	10	-	Spranghers *et al.* 2017
Restaurant waste (vegan)	prepupa	38.1	43.1	38.6	6,7	2.7	-	Spranghers *et al.* 2017
Biogas digestate	prepupa	38.6	42.2	21.8	5,6	19.7	-	Spranghers *et al.* 2017
Vegetable waste	prepupa	41	39.9	37.1	5,7	2.7	-	Spranghers *et al.* 2017
Fruit waste	prepupa	28.3	30.8	40.7	5.6	7.2	-	Meneguz *et al.* 2018
Vegetable & Fruit waste	prepupa	22	41.9	26.3	6.2	13	-	Meneguz *et al.* 2018
Winery by-products	prepupa	26.5	34.4	32.2	5.3	14.6	-	Meneguz *et al.* 2018
Brewery by-products	prepupa	29.1	53	29.9	1.42	7.3	-	Meneguz *et al.* 2018

The crude protein (CP) content in BSF larva ranges from 31 to 63 %, depending on feed substrate. The lowest CP content was reported for larva fed fruit waste and the highest CP for larva fed liver (Meneguz *et al*. 2018; Nguyen *et al*. 2015). It appears that substrates with higher CP and moisture produce larva with higher CP (Oonincx *et al.* 2015; Meneguz *et al.* 2018). The CP also varies during larval growth, decreasing as the larva grows, stabilizing at mature larva to late prepupa and then increasing in pupal and peaking in adult stage (Liu *et al.* 2017).

Furthermore, some authors suggest that using the nitrogen-to-protein conversion factor (Kp) of 6.25 may lead to an overestimation of the crude protein content due to the presence of nitrogen in insect cuticle, digestibly unavailable (Janseen *et al.* 2017; Diener *et al.* 2009; Meneguz *et al.* 2018). Janseen *et al*. (2017) proposed a Kp value of 4.76 for the quantification of protein content in whole larva and a Kp of 5.60 for the protein extracts derived from insects.

Fat content in BSF larva was also reported to vary with diet, ranging from 21 to 41%. For example, BSF larva fed fruit waste contained the highest fat content, and the lowest was observed for larva fed biogas digestate (table 2). These differences could be explained by the fact that insects can synthetize fat from non-fiber carbohydrates, which were almost absent in the biogas digestate (Spranghers *et al.* 2017). On the other hand, there is also influence of the larval development phase where fat content gradually increases through larval development reaching the highest levels in mature larval and prepupal phases (Liu *et al*. 2017).

The dry matter content of fresh larva is high, raging between 22 and 41% (table 2). Bearing in mind the larval development, the content of dry matter raises gradually until the early prepupa stage, and then declines after the mature larva and early prepupa, reaching its lowest in the adult stage (Liu *et al*. 2017).

The ash content shows the largest variation, ranging from 2.7 to 19.7% and seems to be strongly correlated with the ash content in the diet consumed (Spranghers *et al*. 2017).

Chitin content varies between 1.4 % and 6,7%. Some authors suggest that it seems to be influenced by larva development time (Diener *et al.* 2009; Meneguz *et al.* 2018). For instance, small larva developing in longer times showed a higher level of chitin comparing to heavy larva developing in short times (Meneguz *et al.* 2018).

Table 3. Amino acid content (% DM) of BSF larva reared on different substrates and soybean meal

Amino acid (% DM)	Newton et al., 1977	Newton et al., 2005	Spranghers et al., 2017				Smetana et al. 2016	Sotak-Peper et al. 2017
Feed substrate	Cattle Manure	Swine Manure	Chicken feed	Biogas digestate	Vegetable waste	Restaurant waste	BSF defatted 64%	Soybean meal 47%
Essential AA								
Arginine	2,24	1,77	2,03	2,03	2,00	1,99	3.25	3,41
Histidine	1,91	0,96	1,36	1,35	1,24	1,38	2.13	1,21
Isoleucine	1,96	1,51	1,72	1,84	1,73	1,91	2.37	2,13
Leucine	3,53	2,61	2,86	2,95	2,80	3,06	3.68	3,62
Lysine	3,37	2,21	2,34	2,57	2,26	2,30	3.23	3,03
Methionine	0,86	0,83	0,76	0,87	0,76	0,71	1.04	0,63
Phenylalanine	2,20	1,49	1,70	1,87	1,63	1,64	2.27	2,30
Threonine	0,55	1,41	1,64	1,68	1,54	1,62	2.15	1,77
Tryptophan	0,20	0,59	0,67	0,62	0,58	0,54	-	0,70
Valine	3,4	2,23	2,41	2,49	2,48	2,82	3.68	2,26
Non- essential								
Alanine	3,69	2,55	2,52	2,43	2,42	2,78	3.85	1,98
Aspartic acid	4,56	3,04	3,78	3,36	3,59	3,69	4.16	5,14
Cystine	0,06	0,31	0,25	0,24	0,21	0,22	1.60	0,62
Glycine	2,88	2,07	2,26	2,26	2,22	2,52	3.70	1,93
Glutamic acid	3,81	3,99	4,19	3,98	4,13	4,58	4.48	7,14
Proline	3,26	2,12	2,25	2,21	2,14	2,51	3.20	2,26
Serine	0,12	1,47	1,66	1,55	1,50	1,59	2.21	2,13
Tyrosine	2,51	2,38	-	-	-	-	3.49	1,68

The most representative essential amino acids (AA) in BSF larva protein are lysine, leucine, arginine and valine. If BSF would be defatted to obtain oil and protein meal, crude protein levels would be higher, reaching up to 60% (Surendra *et al* 2016). Consequently, amino acid composition of BSF protein meal would be superior that of soybean meal (Newton *et al.* 2005; Spranghers *et al*. 2017; Surendra *et al* 2016).

The amino acid content of BSF larva does not differ much between substrates as some of the other nutritional components (Spranghers *et al*. 2017). Nonetheless, with BSF reared in manure substrates the AA contents vary in accordance with the manure type, showing a tendency to be slightly higher in larva fed cattle manure than in larva fed swine manure (Newton *et al.* 2005; Barragan Fonseca *et al*. 2017). However, it is important to note that processing methods can affect the amino acid content. For instance, killing by freezing activates enzymatic pathways that result in the loss of some AA such as lysine, methionine and cystine (Leni & Sforza, 2019).

Table 4. Fatty acid content (% of total fatty acids) of BSF larva reared on different substrates and soybean oil

Fatty acid	Sealey et al. 2011		Oonincx et al. 2015		Spranghers et al. 2017		Meneguz et al. 2018		Sanchez-Muros et al. 2014
Feed substrate	Cattle manure	Fish offal	By-products (high fat)	By-products (low fat)	Chicken feed	Vegetable waste	Vegetable and Fruit waste	Brewery by-products	Soybean oil
Lauric acid (C12:0)	23,6	37,1	28,9-38,4	48,4-50,7	57,4	60,9	52,1	34,7	-
Myristic acid (C14:0)	5,1	6,3	7,4-7,8	9,9-9,5	7,3	9,5	10,4	6,7	0.1
Palmitic acid (C16:0)	19,8	17,3	14,4-17	11,6-11,8	9,7	8,7	13,9	20,4	10.3
Palmitoleic acid (C16:1)	6,3	7,6	2,9-3,4	4,7;6,6	2	2,9	3,4	2,9	0.2
Stearic acid (C18:0)	6,5	2,0	2,4-2,8	1,8-2	1,4	1,1	2,6	1,8	3.8
Oleic acid (C18:1 c9)	22,7	18,8	15,9-18,1	10,3-10,8	7,54	5,7	8,5	9,2	22.8
Oleic acid (C18:1 c11)	-	-	-	-	0,2	0,3	0,4	0,6	-
Linoleic acid (C18:2 n6)	6,8	5,9	8,3-17,1	3,6-6	11,6	4,5	7	23,5	51
α-linolenic acid (C18:3 n3)	0.0	0,5	0,8-1,5	0,6-1	0,7	1,4	1,7	2,5	6.8

Across studies, the fatty acid profile of BSF larva has been found to be mainly composed of saturated fatty acids (SFA) raging from 61% to 83% of total fat content. These findings suggest that for the BSF larva, the FA profile is only partially affected by the rearing substrate and is in part species-specific (Spranghers *et al*. 2017; Oonincx *et al.* 2015). In fact, a tendency to accumulate SFA's and MUFA's has been well reported for Diptera species (Ramos-Bueno *et al.* 2016). Some authors suggest that fat accumulation in SFA could be an adaptation of BSF to the subtropical climates because SFA are less prone to lipidic oxidation than UFA, allowing BSF larva to survive at temperatures above 40ºC (Meneguz *et al.* 2018).

Of the SFA's, lauric acid (C12:0) is the major constituent, being found in high concentrations (up to 61% of total fatty acids) in BSF larva, irrespective of very limited amounts in diets (Oonincx *et al.* 2015; Spranghers *et al.* 2017; Meneguz *et al.* 2018). Those authors suggest that it is synthesized by the larva. Dipterans also have typically high concentrations of palmitic acid (C16:0), palmitoleic (C16:1) and oleic acid (C18:1) (Thompson *et al.* 1973). Also, high levels of myristic acid (14:0) and stearic acid (C18:0) were found (Meneguz *et al.* 2018; Spranghers *et al*. 2017).

BSF larva can contain 10 to 19% of monounsaturated fatty acids and 5 to 26% of polyunsaturated fatty acids (Spranghers *et al*. 2017; Meneguz *et al*. 2018). Spranghers et al. (2017) indicated that UFA levels in BSF larva are positively correlated with respective levels occurring in diet. Oleic acid (c18:1 C9) is the main representative MUFA in BSF larva, whereas linoleic (C18:2 n6) and alfa-linolenic acid (C18:3 n3) are the principal representatives of PUFA n6 and PUFA n3, respectively (Meneguz *et al.* 2018). Some studies suggest that some insects namely BSF larva are not able to synthetize PUFA's, and therefore these are most likely to originate from the substrate (Thompson *et al.* 1973; N. Ewald *et al.* 2020). Hence it seems possible to incorporate n 3 fatty acids from the diet into the larval fat as demonstrated by N. Ewald *et al*. (2020) where BSFL fed ensiled mussels contained 8% EPA (20:5 n3) and 5% DHA (22:6 n3).

Several authors suggested that rearing substrates have significant effects on macro and micro minerals of BSF larva (Newton *et al.* 2005; Tschirner & Simon et al 2015; Spranghers *et al.* 2017; Chia *et al.* 2020). Nonetheless, all authors report calcium as the most abundant mineral in BSF, which is in accordance with the reported by Finke (2007) that the BSF larva contain significant amounts of calcium in their cuticle (table 5). The BSF larva are also characterize by high concentrations of phosphorus, potassium, sodium and magnesium. Spranghers *et al.* (2017) in the correlation study suggests that these minerals appear to be unaffected by the rearing substrate, but these appear to vary substantially in manure substrates (Newton et al. 2005) (table 5).

Table 5. Mineral content (% DM) of BSF larva reared on different substrates

Mineral	Newton et al. 2005		Spranghers et al. 2017			
Feed substrate	Poultry Manure	Swine manure	Chicken feed	Biogas digestate	Vegetable waste	Restaurant waste
Macro minerals						
Calcium	5	5,36	2,8	6,6	2,8	0,12
Phosphorus	1,51	0,88	0,5	0,4	0,4	0,4
Magnesium	0,39	0,44	0,26	0,3	0,24	0,21
Sodium	0,13	0,13	0,067	0,089	0,06	0,068
Potassium	0,69	1,16	0,6	0,67	0,59	0,59
Micro minerals						
Iron	0,14	0,08	0,035	0,043	0,001	0,001
Zinc	0,01	0,03	0,016	0,005	0,007	0,007
Copper	0,0006	0,003	0,001	0,001	0,001	0,001
Manganese	0,025	0,035	0,022	0,038	0,024	0,002

Mineral concentrations also change through BSF development. Liu *et al*. (2017) reported significantly higher levels of calcium and phosphorus in early prepupal in relation to the larval stage, pointing that a possible reason is the cuticle formation in prepupal period. Conversely, levels of sodium, iron and zinc were higher in larval than in prepupal stage (Liu *et al.* 2017

The high variability in the BSF nutritional composition can present several challenges, particularly in delivering a product with consistent values of nutrients, but it can also present opportunities, such as manipulating the nutritional value to attend to different objectives, which can be done by using different substrates, harvesting at selected life stages, and processing methods. Nonetheless, further investigations are required to understand the role and the relative balances of the macronutrients (carbohydrates, proteins, lipids) affecting the development, yield and nutritional value of the BSF larva.

4. Pig Nutrition

Currently, the main challenges for industrial pig production are to increase productivity, maximizing the feed efficiency while minimizing production costs and environmental impacts. Thus, it becomes important to adopt dietary strategies and formulate diets that provide the essential nutrients in quantities according to the requirements of the animal, in order to minimize the excretion of nutrients in excess to the environment and at the same time reducing the cost of feed ingredients (Chiba 2013; Pomar & Remus 2019).

The nutritional requirements are the minimal amounts of energy and nutrients such as protein, minerals and vitamins that must be provided to meet the animals' requirements for maintenance and production. Energy and amino acids (protein) are main components of diets and therefore are responsible for most of feed costs.

Usually, two or three primary ingredients are used to provide the bulk of the energy. Carbohydrates are the main source of energy, particularly starch, which is abundant in cereals such as maize, barley and wheat. Energy requirements may also be met by fats and oils (e.g., soy oil and colza oil) which contain 2,25 times more calories than carbohydrates (Reese et al 2000). Besides providing dense sources of energy and essential fatty acids (linoleic acid and linolenic acid for pigs), the use of fats in diets also provide other several advantages such as improved palatability, reduce feed dust and produce low heat increment. Supplementing diets with fat is particularly interesting in the summer, because less heat is produced and allows pigs to consume more feed when intake is reduced (Reece et al 2000). Increasing fat content in diets reduces feed intake and improves feed conversion but it can also increase backfat thickness in growing-finishing pigs (NRC, 2012). Moreover, the fatty acid composition of the dietary fat can affect directly the fatty acid composition of the pig and thereby affecting the quality of the meat (NRC, 2012).

In relation to protein, most of the emphasis in swine nutrition is on essential amino acids (AA) and total nitrogen as a substrate for synthesis of other AA (NRC 2012). The AA are required for body protein synthesis that are used for maintenance and meat or milk production. The 10 essential amino acids that cannot be synthesized by the pig and therefore must be provided in diet are: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Thus, the quality of a dietary protein depends on the ability to provide a proper balance of these amino acids. In general, cereal grains provide between 30 and 60% of the pig's amino acid requirements and because these are notoriously deficient in some essential AA diets are supplement with other sources of protein to ensure proper amounts (e.g., soybean meal) (NRC 2012). For instance, corn is deficient in lysine and tryptophan, and other principal grains such barley and wheat are low in lysine and threonine. For soybean meal, the first limiting amino acid is methionine. In this way, the usual most limiting AA in diets for pigs is lysine followed by tryptophan, threonine and methionine as these are most likely to be at deficient levels after lysine (Chiba 2013). Based on the “ideal protein” concept, which is one that provides all essential AA in balance that exactly match the animal’s needs, the AA requirements for pigs are usually expressed relative to the requirement for lysine, with other amino acids stated in terms of a ratio to lysine=1 (NRC, 2012). Moreover, the AA requirements in pigs differ by stages of growth, these are higher in early starter phases and decreases throughout the growing and finishing phases.

Regarding vitamins and minerals, these are normally poorly available in cereal grains and usual protein supplements. Calcium and sodium are the most deficit minerals and phosphorus although present at high concentrations it occurs mostly (60-75%) in the form of phytate, which is largely unavailable for pigs (Gaudré & Quiniou 2009). Therefore, limestone, salt and phosphates are supplemented in diets. The use of enzymes such as phytase to improve phosphorus digestibility is also a common practice (NRC, 2012).

Since not all of the energy and nutrients contained in feed ingredients are entirely available or used by animals, it is essential to know the nutritional value of feed components. Formulating diets based on available energy and nutrients is a more effective strategy to meet more accurately the animal requirements and to decrease the excretion of unused nutrients on the environment. For that purpose, measuring digestibility coefficients of nutrients and energy values are the most widely used practices to evaluate their bioavailability, and thereby determine the nutritional value of feeds.

In standart digestibility studies, the total collection method of feed nutrients intake and the excreted in feces are performed (Kong & Adeola, 2014). The fraction of nutrients that are not recovered in feces expressed as a percentage of ingested represents the apparent total tract digestibility coefficient (ATTDc). Moreover, measuring the nutrient urine output gives the nutrient balance. The digestibility coefficients vary with age, genetics and animal species, as with the concentrations of nutrients and their interactions.

The energy density of the diet regulates the voluntary feed intake, subsequently regulating the intake of other essential nutrients (e.g., amino acids), so it is of great importance to estimate precisely the digestible energy value of feed ingredients. The energy systems utilized for pigs are digestible energy (DE), metabolizable energy (ME) and net energy (NE). These are indirectly estimated by sequential subtractions of energy loss in feces, urine, gases and heat increment during metabolism, from the energy in the diets or ingredients (Kil et al. 2013). Due to practical purposes of estimating these values the DE and ME are the most utilized systems.

For evaluating the quality of a dietary protein is important to know the composition and digestibility particularly of the essential amino acids (AA) because their availability for protein synthesis will depend on the quantities that are absorbed from the intestinal tract. The absorption of AA occurs in the small intestine of the pig. The undigested AA that passes the distal ileum enter the large intestine and are subjected to fermentation (both catabolism and synthesis) by the microflora with little nutritional significance to the pig. Hence, the AA that have disappeared from the small intestine before passing the distal ileum give a better estimate of AA that been absorbed and might be available to the pig than those excreted in feces. Hence, the preferred method to evaluate the AA digestibility in pigs is the apparent/standart ileal digestibility essay, that involves the collection of the digesta at the distal portion of the ileum (NRC, 2012).

For the macro minerals, calcium and phosphorus are the most studied and because their availability is variable amongst feed ingredients the ATTD of phosphorus and calcium are also performed to allow a greater precision in meeting the needs. (NRC, 2012, page 195).

In conclusion, several strategies can be used with the aim to optimize nutrient use by pigs and therefore reduce environmental load. These include the use of ideal protein concept, supplement diets with synthetic amino acids, formulating diets based on nutrient digestibility, selecting ingredients with superior digestibility coefficients and diet supplementation with exogenous enzymes targeted to improve nutrient digestibility.

4.1 The use of BSF as a feed ingredient

The BSF biomass has been widely studied as a substitute for the conventional protein and oil sources in monogastric animal feeds. Investigations have been carried in several fish species for aquaculture production, poultry (broilers and laying hens) and to a lesser extent in swine. These have focused on the effects on growth performance, palatability, digestibility, fatty acid and lipid composition, carcass characteristics and meat quality. The heterogeneity of the results is however dependent on species and specific nutrient requirements, but in general BSF larva can (partially or fully) replace the traditional ingredients at significant inclusion levels without impairing the aforementioned parameters.

The BSF larva meal has been found to have a high and quality protein, with the most abundant essential amino acids being lysine, leucine and valine the least abundant methionine and cysteine (De marco et al. 2015; Cullere et al. 2017; Tan et al. 2020). The BSF larva fat content is high which could be useful in energy dense diets, however due to its richness in saturated fatty acids, these can be reflected in animal tissue composition and therefore affect the quality of the end product as it was reported in some investigations carried in fish and broilers (Li et al. 2016; Renna et al. 2017; Cullere et al. 2017; Schiavone et al. 2017). However, the BSF larva meal can be defatted prior to inclusion, as it was described earlier. Some authors advocate that a partial removal of the fat could be beneficial due to the antimicrobial properties of BSF fat, mainly lauric acid. The medium chain fatty acids are known to possess antimicrobial activity and lauric acid has been found to be particularly active against gram positive bacteria. This could be interesting for the weaning phase of piglets (Spranghers et al. 2017; Spranghers et al. 2018). BSF larva could also be a good source of minerals, particularly calcium and phosphorus.

Furthermore, several investigations reported chitin to have a negative effect on protein and fat digestibility, adversely affecting performance. On the other hand, chitin was also reported to have positive immune effects and to promote the growth of beneficial bacteria and inhibition of the activity of pathogenic microorganisms proving to be a potential substitute for antibiotics (Van huis 2013).

Chapter III – Metabolic trial

1. Objectives

The aim of the present study was to access the nutritional value of full-fat Black Soldier Fly (BSF, Hermetia Illucens L.) larva meal at partial and complete replacement for soybean meal and oil in diets for market pigs. In order to attend to the objective, metabolic trials were performed for analysis of apparent total tract digestibility and metabolic balance of the diets. Individual performance parameters were also evaluated.

2. Materials and Methods

2.1 Rearing of the Black Soldier Fly larva

2.1.1 Rearing unit

The black soldier fly larva for this specific study were produced with a partnership with a Portuguese start-up company EntoGreen. This company has a pilot research unit, located at the Nacional Institute for Agrarian and Veterinary Research (Santarem, Portugal), where it currently stands a colony of the Black Soldier Fly. The production chain of the Black Soldier Fly larva is based on the control of its life cycle previously described (3.1, page 16) divided into reproduction, nursing and fattening phases.

The production unit is divided in an egg production area (reproduction), nursery and a larva production or bio digestion area. In the reproduction area, the prepupae chosen for breeding are placed in pupation containers. After two to three weeks flies crawl out the pupal skin and fly out to a matting zone, where all conditions of luminosity, humidity and temperature are reunited for flies to express their mating behavior, copulate and lay eggs.

In the nursery room, the nursery containers are organized by age and remain under appropriate conditions for egg hatching. The bio digestion area is a separated room where the young larva from the nursery are inoculated into different type of organic substrate and remain until the process of bio digestion is completed.

2.1.2 Production method of the Black Soldier Fly larva

The larva production cycle begins at reproduction area, where egg clusters are laid by the female fly into oviposition specific devices. The eggs are collected to a glass watch dish with minimized handling as every touch or move decreases eggs survival rate, and weighted. Posteriorly, a calculated amount off eggs is inoculated in a nursery container with a food and water source, and placed at the nursery room, which will allow the neonate larva to hatch and start feeding for a period of approximately five days. After that period, the young larva at the nursery are equally distributed into bio digestion containers with an appropriate food and water content and kept in the bio digestion area where they will grow and fatten until they transform into prepupae stage within fifteen days. The larva for this specific study were fed a standard colony diet formulated by EntoGreen.

2.1.3 Harvesting and Post-treatment of the larva

Harvesting is the process in which the larva are separated from the remaining residue. For the present study the harvest was at the prepupal stage. The harvest was performed using an automated shaking sieve (MCL1000, MCLEÇA, Leça da Palmeira, Matosinhos, PORTUGAL) with three sieve mesh sizes of 3, 5 and 7 mm assembled from the largest to smallest, that during the shaking enabled all sized residue particles to fall through the sieves into fertilizer bags and the larva to remain on the top of the sieve or fall through the first and second sieve mesh which were connected to containers where the larva drop into.

Once the harvesting was completed, the larva remain in the containers for some hours, because crawling around helps to clean their skin and gives them time to empty their gut. The separated substrate was stored and processed into fertilizer.

Post-processing of the larva consisted at freezing (-20ºC) for instant killing and storage, followed by defrosting at room temperature before drying in a ventilated oven (60ºC) until constant weight. The dried larva were stored in vacuum bags for posterior inclusion in the diets.

2.2 Proximate analysis and amino acid composition of the BSF larva

Table 6. Chemical composition of the Black soldier Fly larva

DM (%)	96.57
CP (Nx6.25) (%)	40.3
Crude fat/ EE (%)	27.8
Crude fiber (%)	7.4
NFE (%)	18.6
Ash (%)	9.8
Calcium (%)	2.9
Phosphorus (%)	0.98
Sodium Chloride (%)	0.05
Gross energy (kcal/100g)	486.2
Amino acid (% DM)	
Arginine	2.462
Histidine	2.173
Isoleucine	2.116
Leucine	3.015
Lysine	2.746
Methionine	0.703
Phenylalanine	1.681
Threonine	1.540
Tryptophan	0.472
Valine	2.656
Alanine	2.536
Aspartic acid	3.393
Cystine	0.513
Glycine	2.903
Glutamic acid	4.699
Proline	2.232
Serine	1.738
Tyrosine	2.579

2.3 Metabolic trial

The experiment was conducted at INIAV- National Institute for Agrarian and Veterinary Research, located in Santarem, Portugal, in the period between January to March 2020.

2.3.1 Animals, Housing and Experimental Design

The experiment was performed with a total of 12 non-castrated barrows from industrial crossbreeds Pietrain × (Duroc × Large White × Landrace) with mean body weight of 65,52 ± 2,2 kg at the fattening/finishing phase, sourced from a commercial farm (Montijo, Portugal). Upon arrival to the experimental station, the pigs were weighed, identified with a unique number by an ear tag, and allotted into two experimental groups of six animals each with similar bodyweights (64,75 ± 2,36 and 66,29 ± 1,82 kg). The animals were randomly assigned to three dietary treatments, each replicated two times per group (a total of four pigs per treatment).

The experiment was conducted in a pavilion with ventilation trough tilting side windows, equipped with ten individual concrete floor pens (1.35 x 1.80m) provided with individual feeder, and six metabolic cages adjustable lateral and longitudinally to the size of the animal, each one equipped with a feeder in the front and with specific collectors for urine and feces at the lower and back part.

In the course of the experiment, the two groups alternated between individual pens and metabolic cages over periods of seven days, for the control of feed intake and separated sampling of feces and urine. The trial lasted five weeks per group of pigs (three weeks at the metabolic cages and two weeks at the individual pens).

2.3.2 Experimental Diets

Three experimental diets for growing-finishing pigs were formulated by TECADI-Industry and Trade of Products for the Agri-Food Sector, Lda (Santarem, Portugal). The control dietary treatment contained soybean meal (SBM) with 44% of crude protein and soybean oil. For the other two treatments soybean meal and oil were replaced either partially (50%) or completely (100%) with full fat BSF larva meal (BSF50 and BSF100, respectively). Maize meal, wheat and barley were included as energy sources. Adjustments were made in the ingredient composition in order to maintain similar calculated values for energy and crude protein (15% CP).

The ingredients and additives were obtained at the experimental compound feed unit of INIAV in order to produce one tone for each diet treatment. The ingredients of the experimental diets and their relative inclusion levels are reported in table 7.

Table 7. Composition of experimental diets, % dry matter.

Parameter	Dietary Treatment		
	SBM	BSF 50%	BSF 100%
	Ingredient (%)		
Maize	10	10	10
Wheat	26.6	30	10.6
Barley	39.9	38.7	59.1
Soybean meal 44%	17.6	8.5	0
BSF Larva	0	8.78	17.6
Calcium Carbonate	0.57	0.19	0.08
Dicalcium phosphate 17,5/2	1,6	1.3	0.9
L-Lysine HCl	0.25	0.3	0.33
Methionine	0.15	0.16	0.18
Sodium bicarbonate	0.09	0.09	0.09
Salt	0,38	0.38	0.38
Grain Tec TS	0	0.1	0
LUMANCE Dry (Novyrate C / Adimix PRECISION / Novyrate / Adimix 30 coated)	0,08	0.08	0.08
Vitatec Growing / Finishing pigs	0,4	0.4	0.4
Escent S (Unike Plus Dry)	0.08	0.08	0.08
Betaine HCl	0.15	0.15	0.15
L- Tryptophan	0.003	0.009	0.02
Threonine	0	0.002	0.008
Soybean oil	2.2	0.79	0

2.3.3 Handling

The trial was preceded by a period of seven days of adaption to the new environment and to the previously assigned experimental diets. Each pen and metabolic cage were labeled with a number and diet type matching an animal in particular, changed every week period.

The amount of feed was calculated about 5% of bodyweight and adjusted each week. Pigs were fed *ad libitum* twice a day in equal portions, receiving the diets mixed with drinking water 1:2.

Pig pens were cleaned every day with water and the metabolic cages were cleaned on a weekly basis, at the moment of switching groups. The health status of animals was monitored daily by observation.

2.3.4 Growth Performance records

2.3.4.1 Body weight and feed intake

The animals were individually weighted every week and live body weight recorded. Adjustments in the amount of feed were made, and each animal was changed from its pen to the metabolic cage and vice-versa, to start a new seven-day period of metabolic trial. On the day of weighing, pigs were only fed after weighting.

Every day, thirty minutes after the beginning of feeding, the refusals were removed and posteriorly dried in a ventilated oven and weighted, to take into account in the calculations of feed consumption. Calculations of feed intake were only made for the periods at the metabolic cages.

2.3.5 Sampling

Sample collection was performed in each seven-day period at the metabolic cages, corresponding to three collection periods per group of animals. Feces and urine were daily quantified and sampled in the morning.

The total amount of feces was collected from each cage to individual bags properly labeled, weighed and stored at -20ºC for posterior processing and analysis.

The urine was collected to plastic jars assembled with a funnel and equipped with a filter to prevent contamination with any food scraps or feces and containing 5 ml of hydrochloric acid (HCL) 6N added daily to avoid ammonia loss. The daily urine output was measured with 2000 ml and 200 ml beakers, and samples of 10% total amount were taken and stored into individual labeled containers at -20º C for posterior analysis.

During each collection period a sample of each diet was taken every day to obtain a pooled sampled for chemical analysis.

2.3.6 Laboratory analysis

All chemical analysis were performed at the laboratory of nutrition at INIAV, following the methods described by the European and Portuguese Normatives.

2.3.6.1 Feed

All samples of experimental diets were grounded using a 1 mm sieve before analysis. Samples were analyzed for dry matter content by oven drying at 103 ºC to constant weight (NP 875:1994, method ISO 6496:2018); total nitrogen content was determined by Kjeldahl method (NP 2030:1996, method ISO 5983-2:2009) and crude protein was determined multiplying total nitrogen with 6.25 conversion factor; ether extract, a measure for crude fat, was analyzed gravimetrically after extraction with petroleum ether using a Soxhlet system (NP ISO 6492:2014); crude fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was determined using Van Soest method (ISO 13906:2008;Van Soest et al. 1991); sugar and starch total content (Technic note, INIAV); Gross energy was determined using the bomb calorimeter method (ISO/TC 34/SC 10-N 339:1984). Total ash content was determined by incineration at 550 for 4h in a combustion oven (NP ISO 5984:2014); total calcium content was determined by atomic absorption spectrometry method (NP 1999, method ISO 6869:2000) and total phosphorus content was determined using spectrophotometric method (NP 874:2000, method ISO 6491:1998).

2.3.6.2 Feces

At the end of each sampling period, the total amount of feces produced per animal were individually homogenized using an industrial mixer for five minutes at constant speed. A representative sub sample of approximately 900 grams was taken and posteriorly dried in a forced air oven at 60ºC for 72h to constant weight. All dried samples were grounded using a hammer mill with 1 mm sieve before analysis.

A total of 36 samples, three samples per animal regarding each collection period were analyzed for dry matter, crude protein, gross energy, total ash content, calcium and phosphorus with the methods described above.

2.3.6.3 Urine

Urine sub-samples were analyzed for total nitrogen content, volatile nitrogenous bases content by microdiffusion method (NP 4038:1990), gross energy, calcium and phosphorus.

2.3.7 Calculated parameters

2.3.7.1 Growth performance

The weekly body weights were used to calculate average daily gain (ADG). Feed offered to the pigs and unconsumed portions were used to calculate average daily feed intake (ADFI). Total body weight gain and feed consumed were used to calculate feed conversion ratio (FRC) for each dietary treatment. The average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and body weight gain (BWG) were calculated for each week period at the metabolic cages.

2.3.7.2 Nutrient digestibility and balance

The apparent total tract digestibility (ATTD) was calculated for dry matter (DM), total nitrogen (N), gross energy (GE), phosphorus (P) and calcium (Ca) following the equation:

$$ATTD= [(Fi - Ff)/Fi] \times 100\%$$

Where Fi is the total intake of DM (g), GE (Mj), total N (g), P (g) and Ca (g) during the collection period and in a DM basis and Ff is the total fecal output of DM (g), GE (Mj), total N (g), P (g) and Ca (g) originated from the feed that was fed during the collection period.

The nutrient balance was calculated for total N and GE, following the equations:

Nutrient Balance = Nutrient intake – (Nutrient excreted in feces + Nutrient excreted in urine)

Nutrient Balance (% of intake) = Nutrient intake – (Nutrient excreted in feces + Nutrient excreted in urine) / Nutrient intake

2.3.8 Statistical analysis

The data were analyzed using Proc Mixed from SAS. Effects of diet were tested, included in model as fixed factor. An individual pig was used as the experimental unit to analyze growth performance and nutrient digestibility and balance (n= 4 per treatment with 3 observations per animal). The results were expressed as the least square mean and standart error of the mean and compared using multiple test method. Probability values less than 0.05 were considered statistically significant.

3. Results

3.1 Diet composition

The chemical composition of the three experimental diets is summarized in table 8. Although the diets were formulated to be isonitrogenous and isoenergetic there were variations on some of the dietary contents. The total protein content was higher for the BSF50 diet (17.8% CP) followed by BSF100 diet with 16.9% CP and the lowest value for the SMB diet with 16.2% CP, determining a variation of 1.6% between the highest and the lowest value. The crude fat content also increased as the inclusion of BSF increased, with a variation of 3.3% between the lowest value for SBM and the highest for BSF100. The SBM diet contained higher levels of calcium and phosphorus comparing to the BSF diets. The energy content also increased as the inclusion of BSF meal increased, which determined an increase from 17.9 Mj/kg DM for SBM, to 18.6 Mj/kg DM for BSF50 and 19.3 Mj/kg DM for BSF100 (variation 1.4 Mj/kg). An increase of the NDF content was observed with the inclusion of the larva in the experimental diet.

Table 8. Analyzed chemical composition of the experimental diets

Items (g/kg DM)	Diet		
	SBM	BSFLM50%	BSFLM100%
Dry matter	876	880	886
Crude protein	162	178	169
Crude fat	36	48	69
Crude fiber	45	47	49
NDF	156	164	169
ADF	53	53	53
ADL	9.1	6.6	7.1
Sugar	44	43	30
Starch	574	556	518
Ash	53	51	49
Calcium	9.1	7.6	7.5
Phosphorus	7	5.5	4.8
Gross Energy (Mj/kg DM)	17.9	18.6	19.3

3.2 Growth performance

The growth performance of the pigs is reported in table 9. Body weight gain (BWG) was significantly affected by diet (p= 0.0374). Pigs fed the SBM diet and the BSFL50 had significantly higher BWG than the pigs fed BSF100. Dietary BSF nclusion did significantly influence the ADG (p=0.0384). When fed BSF100, ADG of pigs was significantly lower compared to SMB and BSF50. There was no significant effect of diet in the average daily feed intake (ADFI) and feed conversion ratio (FCR) (p=0.257).

Table 9. Effects of the experimental diets on growth performance of growing - finishing pigs (n=4)

Parameter	Diets			P value
	SBM	BSFLM50%	BSFLM100%	
BWG (kg)	8.78 ± 0. 685^{a}	8.60 ± 0.685^{a}	6.43 ± 0.685^{b}	0.037
ADG (kg/day)	1.26 ± 0.098^{a}	1.23 ± 0.098^{a}	0.918 ± 0.098^{b}	0.038
ADFI (kg/day	2.99 ± 0.097	2.87 ± 0.097	2.86 ± 0.097	0.544
FCR	2.47 ± 0.132	2.48 ± 0.161	2.86 ± 0.212	0.259

Means with different letters are significantly different at $p < 0.05$

3.3 Apparent nutrient digestibility and balance

The intakes, excretions, apparent total tract digestibly (ATTD) and balance of nutrients are reported in table 10. There were no significant differences found between the dietary treatments except for dry matter digestibility ($p= 0.024$) and energy digestibility and balance ($p= 0.007$ and $p=0.004$). Apparent digestibility of dry matter for pigs fed BSF50 was slightly higher than those fed the SBM diet. There were no differences in the gross energy (GE) intake and excretion in feces and urine but ATTD of energy was significantly higher for the BSF diets. The energy balance when expressed as a percentage of intake was significantly higher for the BSF diets. There was no significant effect on calcium apparent digestibility amongst diets, although the intake and excretion were higher for the SBM ($p=0.002$ and $p=0.0067$). Similarly, phosphorus intake was significantly higher for the SBM diet ($p=0.001$) comparing to the BSF diets, and phosphorus excretion in feces was significantly higher for SBM in comparison with the BSF100 diet ($p=0.005$), but no significant differences were observed for the ATTD of phosphorus amongst the diets.

The urine fractionating is present in table 11. There were no significant differences in the urinary ammonia (NH_3) excretion between diets.

Table 10. Intake, Apparent total tract digestibility (ATTD) and balance of the nutrients (DM, Nitrogen, Gross energy, Phosphorus and Calcium) of the experimental diets for growing-finishing pigs (n=4).

Item	Diet			P value
	SBM	BSFLM50%	BSFLM100%	
Dry matter				
Intake, g	2627.8 ± 85.07	2527.9 ± 85.07	2532.55 ± 85.07	0.649
Apparent digestibility, %	86.74 ± 0.404^{b}	88.38 ± 0.404^{a}	87.83 ± 0.404^{ab}	0.024
Nitrogen				
Intake, g	68.20 ± 2.31	71.78 ± 2.31	68.39 ± 2.31	0.478
Fecal, g	9.59 ± 0.59	9.07 ± 0.59	9.22 ± 0.59	0.819
Urinary, g	16.81 ± 1.00	18.11 ± 1.00	15.04 ± 1.00	0.157
Apparent digestibility, %	85.88 ± 0.57	87.44 ± 0.57	86.44 ± 0.57	0.169
Balance, g	41.43 ± 1.33	43.19 ± 1.76	44.53 ± 0.92	0.111
Balance, % of intake	60.96 ± 1.55	62.04 ± 1.55	64.57 ± 1.55	0.256
Gross energy				
Intake, Kj	47051 ± 1582.55	47293 ± 1582.55	48769 ± 1582.55	0.769
Fecal,	6613.08 ± 354.65	5805.58 ± 354.65	6138.08 ± 354.65	0.285
Urinary,	928.27 ± 66.75	1085.74 ± 66.75	1000.67 ± 66.75	0.264
Apparent digestibility, %	85.83± 0.45^{b}	87.94 ± 0.45^{a}	87.33 ± 0.45^{a}	0.007
Balance, Kj	39510 ± 1269.26	40402 ± 1269.26	41630 ± 1269.26	0.645
Balance, % of intake	83.83 ± 0.49^{b}	85.51 ± 0.49^{a}	85.28 ± 0.49^{a}	0.044
Calcium				
Intake, g	24.01± 0.84^{a}	18.99 ± 0.84^{b}	19.04 ± 0.84^{b}	0.0002
Fecal, g	13.79 ± 1.27^{b}	9.16 ± 1.49^{a}	7.85 ± 1.19^{a}	0.007
Apparent digestibility, %	47.19 ± 4.14	55.09 ± 3.88	57.53 ± 3.69	0.177
Phosphorus				
Intake, g	18.32 ± 1.23^{a}	13.99 ± 1.23^{b}	12.86 ± 1.23^{b}	0.009
Fecal, g	6.27 ± 0.35^{a}	5.29 ± 0.35^{ab}	4.51 ± 0.35^{b}	0.005
Apparent digestibility, %	59.17 ± 2.31	63.21 ± 1.9433	63.78 ± 1.94	0.221

Means with different letters are significantly different at $p < 0.05$

Table. 11 Urine fractionating

	SBM	BSFLM50	BSFLM100	P value
NH3 excreted urine	2.63 ± 0.026	3.45 ± 0.026	2.78 ± 0.026	0.068
NH3 excreted urine (% total N excreted)	16.14 ± 1.06	19.66 ± 1.06	17.92 ± 1.02	0.095
NH3 excreted (% of N total ingested)	3.91 ± 0.29	4.81 ± 0.29	4.06 ± 0.29	0.071

4. Discussion

The research on the use of BSF larva as a protein source and energy source in diets for monogastric species has been increasing and showing promising results. However, the information available on the utilization of BSF larva meal in the diets for pigs is scarce. The few experiments available have focused mostly on weaned piglets and using low levels of inclusion (until 10%) of BSF in replacement for the traditional protein sources (fishmeal, soybean meal and dried plasma).

The aim of the present study was to access the nutritional value of non-defatted BSF larva meal in partial (50%) and total (100%) replacement of soybean meal and oil in growing finishing pigs (9 and 18% inclusion levels). The main objective was the evaluation of the digestibility and balance of nutrients, although the growth performance parameters were also evaluated.

Our results suggest that the growth performance of growing finishing pigs fed diets with BSF larva meal can be comparable to those fed soybean meal diets. However, it is important to note that performance data collected in this study was only respective to the periods at the metabolic cages and did not cover the whole experimental period, therefore assumptions made from this data do not reflect the normal conditions of pig production. Also, the number of animals used in this study (n=12) limits the relevance of the data for performance parameters due to individual variability.

Nonetheless, it was noted that at a total level of replacement (BSF100) pigs showed a decrease in the BWG and ADG (table 9). To the best of the author's knowledge there is only one study that evaluates growth performance parameters in growing-finishing pigs using high levels of inclusion of BSF larva (9, 12, 14.5, 18.5%). In contrast with our results, Chia *et al.* (2019) reported no effects on ADG or total BWG when replacing fishmeal with BSF meal at total replacement levels (18.5% inclusion level) in growing-finishing pigs. Moreover, our results showed no effects of diet on the FCR, which is consistent with Chia *et al* (2019) that reported that substitution of 75% and 100% with BSF had no significant effects on FCR. The ADFI was not affected by diet indicating that the BSF is as palatable as SBM, which has been also documented in other investigations (Newton et al. 1977). Moreover, another study in finishing pigs with lower inclusion levels of BSF larva meal (4% and 8%) reported that the 4% inclusion resulted in a significant increase in the final BW and ADG and a decrease in FCR comparing to the SBM and 8% BSF diet (Yu et al. 2019).

In weaning pigs, some authors reported that the inclusion levels of full-fat (4 and 8%) and defatted (5.4%) BSF (Spranghers et al. 2018), and inclusion levels of 3.5% BSF (Driemeyer 2016), and BSF in substitution for 5 or 10% soybean meal (Biasato et al. 2019) did not affect growth performance parameters. Moreover Newton et al. (2005), reported that mixing 2.5% BSF larva meal with 2.5% dried plasma resulted in a slightly better performance (+4% gain and 9% feed efficiency) comparing to the control diet (5% dried plasma). Similarly, Yu et al. (2020) also reported an inclusion of 2% BSF to affect positively the performance of piglets, referring an increase in BW and ADG and decrease in FCR during the earlier period comparing to the control diet.

The variability of data can be explained by different dietary inclusion levels of BSF, nutritional properties of the BSF used in each study, which can vary substantially in accordance with the rearing substrate, stage at harvest and processing methods.

Measuring digestibility is a means to estimate the nutrients that are absorbed and may be available to the animals. Moreover, it gives valuable information in order to determine the suitable levels of inclusion and obtain precise values for diet formulation (Veldkamp 2015). To the best of the author's knowledge this is the first study evaluating the digestibility of nutrients of dietary inclusion of BSF larva meal at higher inclusion levels in growing finishing pigs. However, investigations have been carried in piglets showing different results.

Biasato et al (2019) showed that replacing soybean meal with 5% and 10% of BSF larva meal could be done without affecting the digestibility of dry matter, crude protein and ether extract. On the contrary, Yu et al. (2020) showed that inclusion levels until 2% of BSF larva meal were possible but at 4% of inclusion resulted in a significant decrease in crude protein and fat digestibly, however no significant effects were observed for dry matter digestibility. Newton et al. (1997), in a study carried in piglets fed a soybean meal diet compared with a BSF larva diet at 33% inclusion level, reported a significant higher ATTD of dry matter (85% vs 77%), ash (61 vs 45 %), NFE (91 vs 84%) for the SBM diet than for the BSF larva diet. However, ATTD of nitrogen was similar between diets (77 vs 76 %), and the BSFLM diet showed an improved digestibility of ether extract (84% vs 73%).

In the current study, the dietary inclusion of BSF at partial replacement resulted in a higher ATTD of dry matter (88%) comparing to the SBM diet (86%) (table 10). The partial and complete replacement of SBM for BSF also resulted in a slight improvement on digestibility of gross energy (87% vs 85%). The digestibly of nitrogen was similar for SBM and BSF diets (85% vs 87%).

The BSF larva used in the present study contained 27.8% of fat which in turned resulted in the BSF diets containing more fat than the SBM diet (table 8). Thereby, although the digestibility of fat was not evaluated in this investigation it could explain the higher digestibility of gross energy for the BSF diets. Fats and oils are rich energy sources and also have a high digestibility (~85%). Usually, the fat from vegetable sources is more digestible than from animal sources. Nonetheless, the variability of fat digestibility for pigs depends on several factors particularly the composition of fatty acids from triglycerides and their physiochemical properties such as the chain length and the degree of unsaturation, with the digestibility increasing with an increase on unsaturation and a decrease in the chain length (Noblet 2013). Despite that the BSF larva fat being mainly composed of saturated fatty acids, most of this is composed of lauric acid (C12), a medium chain fatty acid that is highly digestible (~94%) compared to long chain fatty acids present in soybean, which may explain the high digestibility of BSF larva fat.

Another factor responsible by differences in the digestibility of nutrients, particularly protein and fat is dietary fiber. Moreover, the digestibility of dietary fiber depends on the fiber source (fiber properties) processing and age or body weight of the animal (increase with increasing age and BW) (Noblet 2013). As it was mentioned earlier, insects contain significant amounts of fiber that can be measured as crude fiber, NDF and ADF and it has been suggested that fiber in insects is represented by chitin due its similarity to cellulose and because the ADF fraction contains nitrogen (Finke 2007). The BSF larva used in this study contained 7.4% of crude fiber, but chitin content was not calculated. In this study we didn't found differences in the ADF fraction of the experimental diets. However, an increase on the NDF content was observed with the inclusion of BSF.

Some studies have reported that chitin may have affected the digestibility of fat and protein resulting in a reduced growth performance. Yu et al. (2020) reported that a BSF larva meal containing 4.5% of chitin (used the ADF fraction as an estimate of chitin), at a 4% inclusion level in diet affected digestibility of crude protein and fat in piglets. In fact, Marono et al. (2015) on a digestibility *in vitro* trial reported a negative correlation between crude protein digestibly and ADF and chitin contents for BSF larva meal.

Furthermore, chitin may also have a nutritive function of energy intake if its digested (Kroeckel et al. 2012; Sanchez-Muros et al. 2016). The breakdown of chitin involves the enzymes chitinase and chitobiase, which has been described to be secreted by the gastric mucosa and pancreas of pigs (Jeuniaux & Cornelius, 1997), however no investigations have been carried about the digestibility of insect chitin for pigs. Nonetheless, a study feeding chito-oligosaccharide from crab shells to piglets showed an improvement in feed efficiency and was found to reduce the growth of harmful bacteria (Han et al. 2007).

To what extent the chitin presented in BSF larva in this study may have affected digestibility of nutrients remains unknown.

In addition, although there was not statistically significance on calcium digestibility probably as a result of a large variation in data, an increasing trend on the ATTD of calcium with the BSF larva meal inclusion can be observed, followed with a decrease on calcium excretion (table 10). This suggests that BSF larva could be a good source of calcium for pigs. Similarly, phosphorus digestibility was also not statistically different between diets, though it showed a trend of decreased excretion and increased digestibility with the BSF larva inclusion (table 10).

Nitrogen excreted in urine is mostly in the form of urea which can be easily converted into ammonia. Whereas amino acids that are not digested are excreted as fecal microbial nitrogen, amino acids that are absorbed and not required for any specific functions are catabolized and excreted as urinary urea nitrogen (NRC 2012). In the present study no significant differences were observed for ammonia urinary excretion between the dietary treatments.

This research did not evaluate the digestibility of individual amino acids. The digestibility of amino acids in pigs is usually measured by the apparent/standart ileal digestibility essay (AID/SID), that involves collecting the digesta at the distal portion of the ileum. Tan et al. (2020) evaluated the ileal digestibility of AA for a BSF larva meal (43.2% CP; 35.5% EE) in growing pigs, showing digestibility values for essential AA of more 74%, with the SID of lysine of 77.6%, threonine 79.8% and methionine 91.8%. Other study (non peer reviewed) Kortelainen et al. (2014) in growing pigs fed two BSF meals – one with fat extraction mechanically (62.9% CP; 18.5% EE) and other with solvent (hexane) extraction (CP 70.5%; 9% EE) - reported the ileal digestibility of AA to be higher in mechanically extracted BSF meal than in solvent extracted (81.9-94.8% and 64-81.8%, respectively). The SID of lysine (81.3%), threonine (82.5%) and methionine (90.7%) for the mechanical extracted BSF larva meal were higher than for the hexane extracted: SID lysine (77.2), threonine (64%) and methionine (81.8%).

The differences across the aforementioned investigations may regard to the nutrient contents of the BSF larva meals, particularly to different levels of fat and protein, and the processing methods to obtain the meals. It is documented that processing methods (e.g., heat treatment, pressure) can affect the digestibility of AA. For instance, excessive heat treatment (temperature, time of heat) may denature the structure of proteins subsequently affecting the digestibility and metabolic utilization of AA (NRC, 2012). The fat content may also affect the digestibility of AA, where some investigations have reported an increase in the SID of AA with increasing dietary fat (Li & Sauer, 1994).

5. Conclusion

The present research has shown that non-defatted Black soldier fly larva meal can be a suitable energy (fat) and protein source for pigs in the growing-finishing period. The BSF larva meal showed to be a promising feed ingredient (up to 18% inclusion level) at complete replacement for soybean meal and oil. Despite that performance parameters at complete replacement showed drawbacks, the digestibility of nutrients did not show any differences between the diets, in fact resulted in slight improvements. For more conclusive data on the growth performance parameters a study containing a larger number of animals should be performed.

Additionally, it is worth mentioning that the BSF meal has several environmental advantages particularly in its production, through the use of local by-products with little or no value for human or animal consumption, reintroducing them back into the food chain as high-quality nutritional sources.

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