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Nutritional value of some insects and their impact on post weaning diarrhoea caused by *Escherichia coli*

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Näringsvärde hos vissa insekter och dess inverkan på avvänjningsdiarré orsakat av *Escherichia coli*

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Abstract

Insects like Black soldier fly *(Hermetia Illucens)*, Mealworm larvae *(Tenebrio Molitor)* and House cricket *(Acheta Domesticus*) are of interest as possible sources of protein to be fed to monogastic animals like pigs and poultry, as they are all thought to have nutrient compositions similar to soybean- or fish meal. This is of interest as the demand for high quality protein is increasing in time with the growing world population. Black soldier fly have been found to have high palatability when fed to pigs whilst mealworm larvae have been found with the ability to utilize substrates of low nutritional quality especially well. House crickets are of interest for being relatively easy to rear and for being palatable. The insects' exoskeleton is partly composed of chitin, which is a polymer similar to cellulose that has been found to potentially affect the ability to digest and utilize proteins negatively. By using chitinous derivates in the feed, it might be possible to reduce the occurrence of post weaning diarrhoea (PWD), which can strike piglets as they are in the process of building up their own microbial flora and immune system. The diarrhoea is commonly caused by specific strains of *Escherichia coli (E.coli)* and the enterotoxin it produces. This is usually treated with antibiotics in the EU. By feeding piglets with chitin or chitinious derivates, the intestinal microbiota of the piglets could potentially be strengthened and the occurrence of enterotoxigenic *E.coli* could potentially be reduced. In order to further evaluate the potential of insects as a source of protein for monogastric animals, as well as determining the function of chitin regarding the intestinal microbiota in piglets, more research is needed.

Keywords: Black soldier fly, House cricket, Mealworm larvae, chitin, chitosan

Sammanfattning

Insekter såsom Svart soldatfluga (*Hermetia Illucens*), Mjölmask (*Tenebrio Molitor*) och Hussyrsa (*Acheta Domesticus*) skulle potentiellt kunna vara alternativa proteinfodermedel till enkelmagade djur som grisar och kycklingar, då de har näringsvärden som liknar soja- eller fiskmjöl. Detta är av intresse då efterfrågan på protein med hög kvalitet ökar i takt med världsbefolkningen. Svart soldatfluga har visat sig ha hög smaklighet när det används i grisfoder och medan mjölmask har visat sig lämpad för att kunna ta tillvara på och använda substrat med näringsfattigt innehåll. Hussyrsan är av intresse på grund av att den är relativt lätt att föda upp och har visat sig ha hög smaklighet. Exoskelettet hos insekter består delvis av chitin, vilket är en polymer liknande cellulosa som troligtvis kan ha negativ påverkan på förmågan att bryta ner och ta tillvara på proteiner. Genom att använda derivat från chitin i fodret skulle det möjligen gå att minska förekomsten av avvänjninngsdiarré (PWD), som drabbar griskultingar när deras tarmflora och immunförsvar är under uppbyggnad. Diarrén orsakas vanligtvis av specifika stammar av *Escherichia coli* (*E.coli*) samt dess enterotoxin. Detta behandlas normalt sett med antibiotika inom EU. Genom att tillsätta chitiin eller derivat från chitin i fodret, skulle tarmfloran möjligtvis kunna bli mer motståndskraftig och förekomsten av enterotoxisk *E.coli* skulle möjligtvis kunna minska. För att ytterligare kunna utvärdera insekters potential som proteinfodermedel, samt chitins funktion gällande tarmfloran hos griskultingar behövs mer studier göras.

Nyckelord: Svart soldatfluga, Hussyrsa, Mjölmask, chitin, chitosan

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1 Introduction

In time with the growing world population, the consumption and the demand for animal products is increasing. This puts pressure on the environment concerning the global capacity as well as on the food- and feed producers (Veldkamp & Bosch, 2015). Soybean- and fish meal are common sources of protein fed to production animals such as pigs and poultry, but the supply has a limit and the costs for purchasing the feed is high (Makkar *et al.*, 2014). Over 80% of the soybeans produced globally today are used for animal feed (Kupferschmidt, 2015). These products could be used for human utilization instead, which makes it relevant to find a source of high quality protein not dependant on soybean- or fish meal that could be used as animal feed. Some insects are thought have a similar composition to soybean meal, which suggests that some insects could potentially meet the nutritional requirements of pigs in the same manner as soybean meal does (Finke, 2002). It has also been found that some insects could hold bacteriostatic substances, such as the polymer chitin, which could potentially reduce and inhibit further development of pathogenic bacteria causing post weaning diarrhoea (PWD) in piglets (Veldkamp & Bosch, 2015). The composition of the insects gives an opening where the bacteriostatic substances might be beneficial, and chitinous derivates could potentially enhance the digestibility of nutrients which could improve the intestinal microbiota as well as the daily growth of weaned piglets (Xu *et al*., 2014).

In the wild, natural weaning takes place when the piglets are around 70 days of age (Whittermore & Green, 2001). According to EU legislation conventional weaning is allowed when piglets have reached an age of 21 days, but depending on the country the age varies (Jordbruksverket, 2017). The process is often linked to a lower intake of feed, a reduction in growth and PWD as a result of the piglets being more easily affected by pathogenic bacteria during the immediate period after weaning (Berkeveld *et al*., 2014). This usually has to do with the piglets' being vulnerable as their immune system and gastrointestinal microbiota is being developed, whilst also experiencing stress as they are weaned (Yao *et al*., 2013). Most cases of PWD are caused by pathogenic strains of *Escherichia coli* (*E.coli*) which affects the gastrointestinal tract (GIT) (McDonald *et al*., 2010). In the EU, this is commonly treated with antibiotics (Bosi *et al*., 2011; EMA & EFSA, 2017). Although effective, the risk of antibiotic resistance is growing with the continuous use (Davis *et al*., 2004).

The potential benefits of using insects in a pig's diet together with the importance of finding a new source of high quality protein for pigs and piglets is vital due to both environmental and economic factors. The production of soy consumes an abundance of agricultural land and water, as well as producing greenhouse gases such as CO_2 (Dalgaard *et al.,* 2007). By rearing insects for animal feed, the production of greenhouse gases and the utilization of natural resources could potentially be reduced due to insect rearing being more effective in terms of the space needed for the production (Kupferschmidt, 2015). Insect rearing could also be environmentally beneficial due to insects' high feed conversion efficiency (van Huis, 2013).

The aim with this review was to look into the nutritional possibility of implementing insects as a source of protein for pigs and to look into some suitable insects for the cause. The review will also look into chitin and its value in regards to *E.coli* causing post weaning diarrhoea in piglets.

2 Literature review

2.1 Gastrointestinal development for piglets during weaning

During the post-natal and weaning process, big changes take place in the GIT of piglets (Yao *et al.*, 2013). They are at their most exposed state in regards to their immune system and the risk of becoming infected by pathogenic bacteria (Bauer *et al.*, 2006). Their immune system is not fully developed when they are first offered solid feed as a complement to milk and there is a distinct change in the microflora during the weaning process (Neu, 2007). At this point, the surface of the GIT and the ability to absorb nutrients increases as the intestinal cells evolve and differentiate to be able to absorb the nutrients in the food (Neu, 2007). According to a study made on mice, it was found that the natural microflora is crucial for the innate immune system and immune cells as they are dependent on the microorganisms for functions regarding repair and protection (Rakoff-Nahoum *et al.,* 2004). If the microflora is not intact or has not developed enough, pathogenic strains of bacteria might afflict the intestine of the weaned piglet. *E.coli* is one of the most common bacteria to cause PWD and it has the ability to infect the intestinal tract and adhere to the walls. The attachment gives the bacteria the opportunity to colonize and multiply causing the infected animal pain and unease (Mc Donald *et al.,* 2002).

It has been found that PWD is a multifactorial disease with *E.coli* and its enterotoxin being one factor, but that PWD is also dependant on the age of the piglets as well as rearing conditions such as pen hygiene, temperature and humidity (Madec *et al*., 2000).

2.1.1 Nutritional requirements

During or after the weaning, as the digestive system is developing, it is common for piglets to experience a decrease in growth performance as they face an appetite barrier, compared to when their diet consisted solely of milk (Cole & Sprent, 2001). This makes it important to find a palatable feed with good digestibility to make sure their feed intake matches their nutritional requirements. It is also important to find a source of protein that not only helps to balance the composition of amino acids, but that could also help to prevent disruption of the intestinal microbiota by having some probiotic property to help the intestinal homeostasis (Cole & Sprent, 2001). The first limiting amino acid for pigs is usually lysine (Henry *et al.*, 1992). When the required level of lysine has been established, other amino acids like threonine, methionine, cysteine and tryptophan are considered in order to achieve a well-balanced diet (Henry *et al.*, 1992). The amino acid balance in feed intended for weaner pigs have been revaluated, as can be found in table 1.

Table 1. The ideal balance of amino acids for weaner pigs. Percentage in relation to lysine Source: Cole and Sprent (2001).

Source	Lysine	Methionine and cysteine	Threonine	Tryptophan
Cole (1978)	100	50	60	18
Fuller *et al.* (1979)	100	53	56	12
ARC (1981)	100	50	50	15
Fuller *et al.* (1987)				
Maintenance	100	150	142	29
Growth	100	53	69	18
Both	100	56	72	19
van Lunen and Cole (1996)	100	50-55	65-57	18

2.2 Chitin and chitosan – favourable components or not?

Chitin is a big part of the insect exoskeleton, with a chemical structure similar to cellulose (Shahidi *et al.,* 1999). Consequently, monogastric animals are unable to digest chitin as they lack the required enzymes. Apart from the exoskeleton, which is the "shell" of the insects, chitin can also be found in algae, yeasts, diatoms and invertebrates and consists of N-acetyl-D-glucosamine units (Synowiecki & Al-Khateeb, 2003). Chitin has the ability to interact with the negatively charged sites of the intestinal wall which changes the permeability and therefore the uptake of proteins and peptides, which suggests that chitin could help the maintenance of the intestinal homeostasis (Proporatto *et al*., 2005). By performing deacetylation of chitin, a nontoxic polysaccharide called chitosan can be derived (Li *et al.,* 2013). This version has a higher digestibility than chitin in its original form (Li *et al.,* 2013).

Several studies have tried to implement chitin or chitinous derivates in animal feed and have come to different conclusions regarding its potentially beneficial properties. Some suggests that chitin or chitosan might have inhibitory properties regarding *E.coli* (Shahidi *et al*., 1999).

In a study by Xiao *et al.* (2014) 30 weaned piglets were observed to determine the effect of chito-oligosacharrides (COS) in the feed regarding diarrhoea caused by enterotoxigenic *E.coli.* The piglets were split into three groups where one group was given an addition of COS, another was given chlortetracycline and the third worked as a control group with nothing added to their corn-soybean meal diet. It was found that supplementing the diet with 300 mg/kg COS had a similar effect to adding 50 mg/kg of the antibiotic chlortetracycline to the feed, in terms of diarrhoea in the piglets. It was also found that there was no significant difference in weight gain between the piglets fed COS and the ones just given the dose of *E.coli* (Xiao *et al*., 2014).

In a study by Khempacka *et al.* (2011) broiler chickens were fed pure chitin as well as shrimp meal containing chitin in different quantities. It was found that the intestinal microbial population could potentially be improved by the addition of maximum 15% shrimp meal with a chitin content of 2,8%, but that higher quantities could decrease the intestinal digestibility. By feeding purified chitin, no improvement regarding intestinal microbes could be found. The study concluded that chitin might be limiting regarding the digestibility of the shrimp meal, but that low levels might increase the appearance of *Lactobacillus* and decrease the appearance of *E.coli* in the intestine (Khempacka *et al.,* 2011).

In a study by Proporatto *et al.* (2005) addition of chitosan to the feed was found to cause improvement in the intestinal and mucosal immunity in activated leukocytes in rats. Another study found that concentrations of serum immunoglobin G (IgG), which is the most common serum antibody, was higher with an increasing amount of chitosan in feed given to weaned piglets (Li *et al.*, 2013). It was concluded that a chitosan dose between 500-1000 mg/kg feed was suitable for achieving the best results regarding the IgG-serum levels. This gave beneficial effects to the humoral immune system, which is crucial for protection against infections caused by viruses or bacteria (McKee *et al.*, 2007).

In another study, chitin was found to increase the amount of bifidobacteria in the gut when used as a food additive to broiler chickens (Austin *et al*., 1981). This phenomenon is commonly known as competitive exclusion which refers to the expansion and adhesion of the bacteria to the intestinal wall that consequently makes it more difficult for pathogenic bacteria to stick to the inside of the gut wall (Hardin, 1960; Shahidi *et al.*, 1999).

2.3 Nutritional value and nutrient composition of insects

The vast majority of the protein based products used globally for animal feed today consists of soybean meal (Hard, 2002). For pigs, the main source of protein comes from soybean meal with a crude protein content between 49 and 56% (CVB, 2007). It has been found that insects in general have a composition of nutrients that could be compared to the nutritional profile of soybean meal. Insects in its larvae state often have a higher amount of fat which usually varies between 10% and 30% of their fresh weight (DeFoliart, 1992). In most cases the fat content is higher in the pulpae and larvae state than in the adult stage of the insect (Chen *et al.*, 2009). If the high fat content is not desirable, defatting the insects could increase the protein values in the final product to an extent that could surpass that of soybean meal (Veldkamp & Bosch, 2015). It has also been found that by altering the feed it is possible to influence the final composition of the insects, whether that is the insect in its larvae, pulpal or adult state (Ramos-Elorduy *et al.*, 2002). The majority of insects have been found to have a composition with high amounts of K, Mg, Fe and Ca, which further supports the idea that some insects might have a nutritional composition that could meet certain requirements of production animals like pigs and poultry (Rumpold, 2013).

In table 2 and 3, comparisons between soybean meal and three insects of relevance regarding amino acid-, mineral- and nutrient composition can be found.

Table 2. Amino acid composition (g/16 g nitrogen) of selected insects, soybean meal and fish meal (Makkar et al., 2014).

Amino acid	Black soldier fly larvae	Mealworm	House cricket	Soybean meal	Fish meal
Methionine	2.1	1.5	1.4	1.3	2.7
Cysteine	0.1	0.8	0.8	1.4	0.8
Lysine	6.6	5.4	5.4	6.2	7.5
Threonine	3.7	4.0	3.6	3.8	4.1
Tryptophan	0.5	0.6	0.6	1.4	1.0

Table 3. Composition of main constituents in selected insects, soybean meal and fish meal (Makkar et al., 2014).

% in DM	Black soldier fly larvae	Mealworm	House cricket	Soybean meal	Fish meal
Crude protein	42.10	52.80	63.30	51.80	70.60
Lipids	26.00	36.10	17.30	2.00	9.90
Calcium	7.56	0.27	1.01	0.39	4.34
Phosphorus	0.90	0.78	0.79	0.69	2.79
Ca:P ratio	8.40	0.35	1.28	0.57	1.56

2.3.1 Black soldier fly (*Hermetia Illucens*)

It has been concluded that black soldier fly larvae (*Hermetia Illucens*) could be of interest as feed for monogastric animals due to its protein and fat composition (Makkar *et al.*, 2015). Although the larvae have a good nutrient composition, additional methionine, cysteine and threonine would have to be added in the feed in order to meet the requirements of pigs (Makkar *et al.*, 2015). In a study it was found that replacing soybean oil with oil from black soldier fly larvae for broiler chickens was possible and no significant difference in palatability, feed intake or growth was found (Schiavone *et al.*, 2016).

In another study where digestibility and palatability was analysed, pigs were given the choice between three separate diets consisting of 20% protein derived from either dried, ground black soldier fly larvae, from soybean meal with added fat or from soybean meal without added fat (Newton *et al.*, 1977). Based on palatability, it was found that the diet based on black soldier fly larvae and the diet based on soybean meal with added fat were possibly exchangeable (Newton *et al.*, 1977). The study showed that using dried soldier fly larvae as a substitute for soybean meal with additional fat gave a lower apparent digestibility of dry matter when it was added to a swine diet fed to pigs at six weeks of age, compared to the diet containing soybean meal without added fat. When offered diets with different composition, the palatability for the larvae based meal was thought to be higher than the diet consisting of soybean meal without added fat, as the pigs did choose the larvae meal in favour of the soybean meal without added fat. The majority chose the soybean meal with added fat above the other choices, which was thought to be an indication that pigs, based on palatability, have a preference for meals with added fat and that soldier fly larvae meal could come to replace soybean meal.

In another study it was found that the fatty acid composition can be altered in the black soldier fly larvae to increase the amount of omega-3 by rearing the flies on fish residue (St:Hilaire *et al.*, 2007). The results of the study concluded that there is a possibility for black soldier fly larvae to replace fish meal as animal feed. In the study by Makkar *et al.* (2014) it was also found that black soldier fly larvae reared on pig manure had an amino acid composition similar to the one of soybean meal, but that both diets lacked in methionine+ cysteine and the larvae meal also lacked in threonine and tryptophan (Newton *et al.*, 1977). According to EU regulations regarding feed safety, rearing on manure is not legal since insects would be classified as “farmed animals”, which is not allowed due to risks of transmission of *Bovine spongiform encephalopathy* (EC 1069/2009). However, it was found in a study that using black soldier flies could be beneficial seeing as it thrives in pig manure which gives the opportunity for a closed orbit in which the animal feed can be produced at the same time as manure is degraded and organic waste can be recycled (Newton *et al*., 2005).

2.3.2 House cricket (*Acheta domesticus*)

House crickets are omnivorous with a preference to be reared at an optimal temperature around 28-30°C (Makkar *et al*., 2014). They have been found to have a high quality protein composition as well as being relatively easy to rear (Finke *et al*., 1989). In one year, they are able to produce up to seven generations and about 2000 crickets can be bred for every m^2 (Makkar *et al.,* 2014). In a study performed on weaning rats it was found that house crickets could be valued equally to soybean based feed in terms of amino acid composition, and that by removing the chitin the quality of the protein was further improved as some of the nitrogen was bound to the chitinous parts in the form of non-protein nitrogen (NPN) (Finke *et al*., 1989). Apart from being nutritious, house crickets are known for their taste which makes them one of the most popular insects for human consumption in Thailand (van Huis *et al.,* 2013).

2.3.3 Mealworm larvae (*Tenebrio molitor*)

Mealworm larvae are omnivorous and can be reared on a mix of plant and animal products as well as on cereal (Ramos-Elorduy *et al*., 2002). Under controlled conditions, they have been found capable of turning substrates with a low nutrient value (8-9% protein) into high quality insect protein (44-61% protein) (Ramos-Elorduy, 1997).

A study was performed by Ramos-Flourdy *et al*. (2002) where mealworm larvae were used as a supplemental protein source in feed for broiler chickens. The study involved diets with 0, 5 and 10% mealworm larvae and no significant deviation between the three could be found regarding weight gain, feed efficiency and feed uptake, which concluded that mealworm larvae could be a potential ingredient in broiler feed (Ramos-Flourdy *et al*., 2002).

In another study, soybean meal was replaced by mealworm larvae meal with the aim to see how it would affect nutrient digestibility, growth and meat quality of broiler chickens (Bovera *et al.*, 2016). Different groups were fed either a soybean based diet or a diet based off of mealworm larvae and it was found that the meat quality was unaffected and that the feed conversion ratio was significantly better in the broilers fed the larvae diet compared to the soybean meal diet. The apparent ileal digestibility of dry matter, organic matter and crude protein for the broilers fed the larvae meal was lower than the other group, which was concluded to be a result of its chitinous content of 4.62%. Chitin has been found to reduce protein digestibility in broiler chickens (Khempaka *et al.*, 2011).

3 Discussion

Based on the information in this literature review, it seems that black soldier fly larvae, house cricket and mealworm larvae do have the capacity to replace soybean- and fish meal in a diet for piglets to some extent, either partly or fully. Black soldier fly larvae meal has been found to have a similar protein composition to soybean meal, but the apparent nitrogen digestibility seem to be lower and the loss of urinary nitrogen in pigs fed the insect meal seem to be higher compared to the pigs fed soybean meal (Newton *et al.*, 1977). This could probably be connected to the chitinous parts of the insect with lower digestibility. Makkar *et al.* (2014) found that black soldier fly larvae meal could be applicable in pig's feed, but that supplementation with methionine, cysteine and threonine would have to be considered due to its deficiency. One option could be to use larvae meal and supplement the diet with synthetic amino acids. Another option could be to replace only a part of the diet with insect meal, to make sure that the nutritional requirements in terms of essential amino acids are met but at the same time limit the use of soybean- and fish meal. To improve the digestibility of the chitinous parts of the insects, deacetylation is an option (Shahidi *et al.*, 1999).

Black soldier fly larvae can be reared on manure from both pigs and poultry, and both black soldier fly larvae and mealworm larvae can be reared on a variety of organic matter and are able to recycle waste products to high quality feed in terms of nutritional value (Makkar *el al., 2014)*. Mealworm larvae have been found especially well-suited for its ability to utilize substrates with a low nutrient content in a favourable manner (Ramos-Elorduy, 1997). This makes them both flexible for factory production as the type of insect, as well as the rearing substrate, can be chosen depending on the nutritional requirements of the pigs, but also depending on what it best suited for the production region wise. This way, the recycling of plant- and animal waste or manure could be a part of a closed orbit system which would bring further control over the production as a whole. This could be beneficial regarding waste handling as well as being cost effective in the end. As of right now, EU-regulations prohibits all use of insects as animal feed (EC 1069/2009). However, this could come to change in the future as insects as a source of protein are of interest in order to meet the demand on food and feed globally.

Studies are lacking regarding house crickets as feed to production animals, but based on their nutritional composition and the fact that they are used for human utilization in Asia, they have potential to be used as animal feed as well. Further studies are needed to determine its value as feed for production animals such as pigs and poultry.

The functional properties of chitin are noticeable in regards to *E.coli* but the effect seems to differ between studies. In the study by Xiao *et al.* (2014) it was found that PWD caused by enterotoxigenic *E.coli* could be reduced by supplementing chito-oligosacharrides to the diets of weaned piglets, but that the weight gain did not differ significantly between the supplemented pigs and the ones just receiving a strain of *E.coli* without treatment. At the same time the digestibility was reduced which could indicate a lesser uptake of nutrients. To be able to implement insects as a prebiotic or antibacterial substances in the feed, more studies have to be performed to be able to target the pathogenic bacteria better.

In the study performed by Li *et al*. (2013) it was found that addition of chitosan to a piglets' diet improved the production of IgG and could therefore indicate a positive response in regards to the piglets' immune system. This further suggests that the right amount of chitinous components in the feed could be of use to the piglets post weaning in order to improve the resistance against enterotoxigenic bacteria. In the study by Finke *et al*. (1989) it was suggested that the protein quality of house crickets could be further improved if the chitinous parts of the insects were removed. In order for these insects to be functional as a source of protein they need to have a nutritional composition that is equal to or exceeds the soybean- and fish meal while simultaneously being palatable to the piglets. This indicates that it might be favourable to separate the chitinous parts from the insects and use insect meal and chitin derivates separately.

As a conclusion, black soldier fly larvae, house cricket and mealworm larvae could potentially be used instead of or in combination with soybean- or fish meal as a source of protein fed to pigs, but black soldier fly larvae seems to be the most promising insect. The containment and function of chitin in the reviewed insects is hard to determine, but since some positive results regarding the piglets' immune system has been found, chitinous compounds could potentially be used to decrease the need for treatment with antibiotics. Although it may not be economically beneficial to begin with, insects as feed could be of great use from an environmental perspective. Further research is needed to determine in what capacity insects can be implemented in the feed to monogastric animals and how derivates from insects can influence the intestinal microbiota of weaned piglets.

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