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APPARENT DIGESTIBILITY COEFFICIENT OF INCLUSION LEVELS OF *Zophobas morio* LARVAE MEAL FOR NILE TILAPIA FINGERLING

LAVRAS - MG

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Orientadora: Prof[a]. Dr[a]. Priscila Vieira e Rosa

Co-orientadora: Prof[a]. Dr[a]. Ana Tereza de Mendonça Viveiros Leal

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APROVADA em 22 de fevereiro de 2019.

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To my mom – Leila and dad – Elair, and to my sisters – Amanda and Bárbara, for all the support, love and trust;

Dedico

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RESUMO

A tilápia do Nilo é a espécie de peixe mais produzida no país, sendo responsável por mais de 58% da produção de peixes do país e quase 36% da produção aquícola em 2017. O farelo de soja é uma das fontes de proteínas mais importantes na dieta des peixes, entretanto, tem alguns fatores antinutricionais, deficiências de aminoácidos e pode afetar a integridade da mucosa intestinal dos peixes, afetando a absorção de nutrientes e digestão então estudos estão sendo feitos em busca de alternativas proteicas para dietas de peixes. Insetos são estudados com esse objetivo por causa da quantidade e qualidade de sua proteína, assim como a sua sustentabilidade. A proteína de *Zophobas morio* varia entre 40 e 68%, portanto nosso objetivo foi estudar o coeficiente de digestibilidade aparente em dietas com inclusão de farinha de larva de *Zophobas morio* em níveis em substituição ao farelo de soja. O objetivo dessa pesquisa foi determinar o coeficiente de digestibilidade aparente dos nutrientes para dietas com inclusão de farinha de larva de *Zophobas morio* para alevinos de tilápia do Nilo. Para isso, 900 alevinos de tilápia foram distribuídos em delineamento inteiramente casualizado em 18 tanques de digestibilidade; alimentados duas vezes ao dia com uma de três dietas experimentais: a controle, com 0% de inclusão de farinha de larva de *Zophobas morio*; 15% de inclusão ou 30% de inclusão. Foi adicionado 0,5% de óxido de cromo como um marcador nessas dietas. As fezes dos peixes foram colhidas durante 15 dias, secadas e depois analisadas para composição centesimal e composição de óxido de cromo. Em seguida, os cálculos de coeficiente de digestibilidade aparente foram feitos segundo a metodologia proposta por Nose (1960) e Cho et al. (1985). Uma substituição total de farelo de soja por 30% de *Zophobas morio* em dietas para alevinos de tilápia não alterou o coeficiente de digestibilidade aparente comparado à uma ração convencional (dieta controle); a inclusão de 15% de farinha de inseto aumentou os coeficientes de digestibilidade aparente dos nutrientes quando comparado à dieta controle.

Palavras-chave: Nutrição de peixes. Farinha de inseto. Coeficiente de digestibilidade aparente.

ABSTRACT

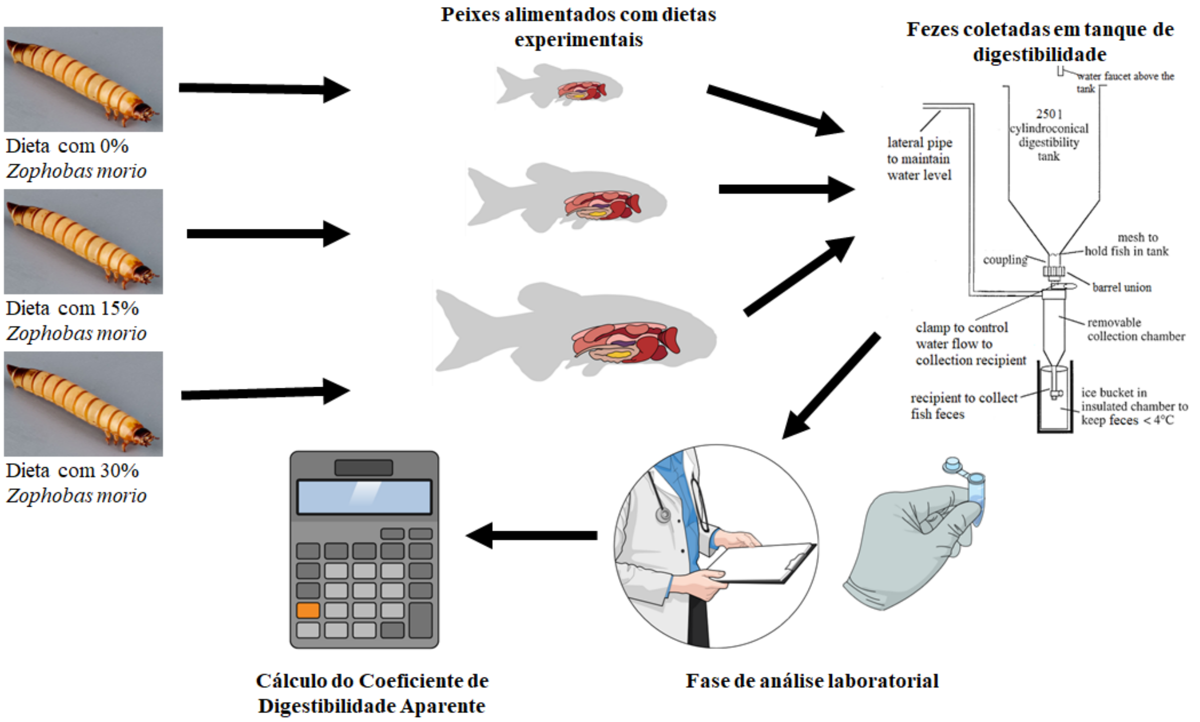
Nile tilapia is the most produced fish species in Brazil, being responsible for over 58% of the country's fish farming and almost 36% of the aquaculture production in 2017. Soybean meal is one of the most important protein sources in fish diet, however, it has antinutritional compounds, amino acid deficiencies and can affect fish intestine's integrity and nutrient absorption and digestibility so studies are being made trying to find an alternative protein source for fish diets. Insect are being studied in this regard because of their protein quality and quantity, as well as their sustainability. *Zophobas morio* protein content varies between 40 and 68%, therefore our goal was to study the apparent digestibility coefficient for diets with inclusion of *Zophobas morio* larvae meal in levels in substitution of soybean meal to find out if this insect can be used in Nile tilapia feed. The goal of this research was to determine the nutrient's apparent digestibility coefficient for diets with Zophobas morio larvae meal in Nile tilapia fingerlings. 900 Nile tilapia fingerlings were distributed in a completely randomized design in 18 digestibility tanks; fed twice a day with one of the three experimental diets: control, with 0% inclusion of Zophobas morio larvae meal; 15% inclusion or 30% inclusion. 0.5% chromic oxide was added as a marker in those diets. Fish feces were collected for 15 days, dried and then analyzed for centesimal composition and chromic oxide composition. Then, the calculations for apparent digestibility coefficient were made based on the metodology proposed by Nose (1960) and Cho et al. (1985). A total substition of soybean meal for 30% Zophobas morio meal in diets for tilapia fingerlings did not affect the apparent digestibility coefficient when compared to a conventional diet (control diet); the inclusion of 15% Zophobas morio meal increased the apparent digestibility coefficient when compared with the control diet.

Keywords: Fish nutrition. Insect meal. Apparent digestibility coefficient.

Coeficiente de digestibilidade aparente em níveis de inclusão de farinha de larva de *Zophobas morio* para alevinos de tilápia do Nilo

Elaborado por **Izabella Luiza Gomes Almeida** e orientada Dra. **Priscila Vieira e Rosa**

Tilápia do Nilo é a espécie de peixe mais produzida no Brasil, é bem aceita pelo mercado consumidor e se adapta bem à diferentes dietas. Um dos ingredientes mais importantes para fornecer proteína nas dietas de peixes é o farelo de soja, mas tem alguns fatores que diminuem a digestão de nutrientes, também tem algumas deficiências de aminoácidos e pode afetar a integridade do intestino, então novos estudos tem sido feitos para substituir farelo de soja na dieta de peixes. Insetos são estudados com esse objetivo por causa da quantidade e qualidade de sua proteína, assim como a sua sustentabilidade. *Zophobas* morio, ou tenébrio gigante, tem nível de proteína alto, portanto nosso objetivo foi estudar o coeficiente de digestibilidade aparente em dietas com inclusão de farinha de larva de *Zophobas morio* em níveis em substituição ao farelo de soja. Foram utilizados 0, 15 ou 30% de tenébrio gigante em substituição ao farelo de soja nas dietas para alevinos de tilápio do Nilo e o óxido de cromo foi utilizado como marcador. 900 alevinos foram distruídos em 18 tanques de digestibilidade, alimentados duas vezes ao dia e as fezes foram coletadas por 15 dias. As composição das fezes e a concentração de óxido de cromo foram analizadas e depois o coeficiente de digestibilidade aparente, que é o quanto o nutriente foi absorvido pelo peixe, foi calculado. Uma substituição total de farelo de soja por 30% de tenébrio gigante não afetou o coeficiente de digestibilidade quando comparado à uma dieta somente com farelo de soja e a utilização de 15% de tenébrio gigante aumentou o coeficiente de digestibilidade aparente, quando comparado à dieta controle.



Representação esquemática de um experimento de digestibilidade em peixes. Peixes são mantidos em tanques de digestibilidade do tipo Guelph modificado e alimentados com as dietas experimentais. As fezes são coletadas e analizadas em laboratório e em seguida, os cálculos são feitos para a obtenção do coeficiente de digestibilidade aparente.

Dissertação de Mestrado em Zootecnia, defendida em 22/02/2019.

SUMMARY

1 INTRODUCTION

With the populational increase and the consumer's demand for healthier food, richer in protein (FAO, 2014), aquaculture is appointed as the next global frontier in food production (SCHULTER; VIEIRA FILHO, 2017). Fish, especially, are indicated to support this demand because their protein has high biological value and fish also have high level of unsaturated fatty acids and vitamins (GONÇALVES, 2011). Fish consumption grows in a rate of 3.2% since 1961, while population growth has been of 1.6%; fish is also one of the most looked-after meats by the consumer, only losing to poultry; fish also was responsible to provide 17% of all animal protein consumed by the world's population in 2015 (FAO, 2018).

Exploratory fishing is decreasing throughout the years and aquaculture production increases. Between 2011 and 2016, total capture decreased in 1,4% while aquaculture increased almost 23%. Continental fish aquaculture reached an increase of almost 25% between those years (FAO, 2018) and Brazil has a huge potential to help increase even more these statistics: FAO (2018) estimates that Brazil aquaculture production will increase by 89% in 2030, and much of it can be attributed to inland freshwater production.

Nile tilapia (*Oreochromis niloticus*) is the most produced freshwater fish species in Brazil, representing 58,4% of the country's fish-farming in 2017 and almost 36% of the aquaculture in the country (IBGE, 2017a). This is due to the wide distribution, adaption, good acceptance of the consumer market (AZEVEDO et al., 2014) as well as its good production characteristics such as being adaptable to a wide range of feed – being an omnivore fish, being docile, having easy reproduction and, especially, having a meat that pleases the consumer, with good organoleptic characteristics and without thorns (SEBRAE, 2014; BOSCOLO et al., 2001).

Brazil is one of the top producers of Nile tilapia in the world (7th place, according to FAO, 2014; 4th place, according to PEIXE BR, 2018) but the production processes can still be improved, one of them being the feed available to Nile tilapia (SCHULTER; VIEIRA FILHO, 2017). Fishmeal is a important feed ingredients in fish diets (FAO, 2018), however, its price continue to rise and the ingredient mainly used as a replacement, soybean meal, does not fall behind either, with increased prices in the past years (FAO, 2013; IndexMundi, 2019), and some nutritional issues as well as antinutritional compounds, amino acid deficiencies (DUMAS et al., 2018) and damage on intestine's integrity, which would lead on losses on nutrient digestibility and absorption (MAHMOUND; KILANY; DESSOUKI, 2014; TRAN-NGOC et al., 2016).

The aquaculture production has been facing pressure to become more sustainable (PEREIRA et al., 2012) and the search for alternative ingredients is growing (DUMAS et al., 2018).

Insects are interesting as an alternative protein source in aquaculture (STAMER, 2015; FAO, 2013) because of the protein quantity and quality in several insect species (SANCHÉZ-MUROS; BARROS; MANZANO-AUGLIARO, 2014). Their amino acid profile is interesting (BARROSO et al., 2014), with some species, such as *Zophobas morio*, being able to reach fish requirement levels, except for methionine (HENRY et al., 2015; BARROSO et al., 2014).

Zophobas morio, also known as superworms, Morio worms or *Zophobas* (VAN HUIS; TOMBERLIN, 2017), characterizes as thick larvae, leathery light brown exoskeleton and dark rings along the body that are more prominent in the encephalic and caudal portions; they hatch measuring up to 2,5 millimeters, usually growing between 2 to 4 centimeters at the time of the harvest (COSTA LIMA, 1955; FERREIRA et al., 2010; VAN HUIS; TOMBERLIN, 2017). According to the nutrients content, *Zophobas morio*- dry matter varies between 38-95%, crude protein between 40-68%, crude fat between 14-40%, NDF between 13-50% and ash 2,7-6,2% (BAKER; FITZPATRICK; DIERENFELD, 1998; JABIR; JABIR; VIKINESWARY, 2012; FONTES, 2018; OONINCX; DIERENFELD, 2011). It is important to point out, however, that these values can alter depending on the insect feed (St-HILAIRE et al., 2007; KROECKEL et al., 2012; HENRY et al., 2015), and developmental stage (NOGALES-MÉRIDA et al., 2018; BARROSO et al., 2014).

As interesting and sustainable that it seems to use *Zophobas morio* in fish diets, some trials must be done in order to determine if they can be considered a good ingredient in feed, and that can be done when determining if this ingredient can be digested by the animal (PEREIRA et al., 2012). A digestibility assay measures the efficiency of digestive and absorptive process of an ingredient (GLENCROSS; BOOTH; ALLAN, 2007) and consists on feeding the animal with a diet containing the ingredient and collecting its feces afterwards (BOSCOLO; HAYASHI; MEURER, 2002); the direct method requires total ingested feed quantification and total feces collection, which is difficult to be done in the aquatic environment (MORALES; CARDENETE; SANZ, 1999), therefore the indirect method, which consists in the incorporation of a marker in the feed and then its determination in both the feed and feces to calculate the apparent nutrient digestibility, is the most used for fish (MAYNARD; LOOSLI, 1969). As for markers, the chromic oxide (Cr_2O_3) is the most used external marker in aquatic animals' assays (VANDENBERG; DE LA NOÜE, 2001). Fish feces collection for digestibility trials in Brazil are mostly done by a decantation column using a modified Guelph system (CHO; SLINGER, 1979; ABIMORAD; CARNEIRO, 2004).

In this work, the goal was to determine the nutrients' apparent digestibility coefficient for diets with *Zophobas morio* larvae meal inclusion as substitute of soybean in Nile tilapia fingerling diets.

2 LITERATURE REVIEW

2.1 Aquaculture

Aquaculture is the man-controlled activity with the goal of productive economic and financial exploration of aquatic creatures. It has been appointed as the next global frontier in food production (SCHULTER; VIEIRA FILHO, 2017). The global demand for fish has been growing in the latest decades due to the population increase and the search for healthier food, richer in protein (FAO, 2014). Fish are protein sources with high biological value, low cholesterol levels and high level of unsaturated fatty acids and vitamins (GONÇALVES, 2011) and as such, the aquaculture stands out as the most interesting alternative to meet this demand of healthy, protein-rich diets, as the fish production has been stable since the 1990 decade (FAO, 2014).

This growth in fish demand can be traced back to 1961. Since then, the average annual increase in global fish consumption has been of 3.2%, while the annual population growth has been of 1.6% (FAO, 2018). Therefore, the interest in fish has grown in a faster pace than the populational increase. Fish consumption has exceeded that of all meats from terrestrial animals combined (2.8% annual increase in consumption for combined terrestrial animals) and exceeded individually for bovine, ovine, pig and others, only losing for the poultry annual increase, that has reached the mark of 4.9%. Per capita, fish consumption grew from 9 kg in 1961 to 20.2 kg in 2015; with 17% of animal protein consumed by the world's population and 7% of all proteins was fish protein in 2015 (FAO, 2018).

Data from FAO, in their The State of World Fisheries and Aquaculture (2018) publication shows that while fishmeal and fish oil still rank as the most nutritious and digestible ingredients for fish feeds, their inclusion percentage in feeds for aquaculture is decreasing, as their use has been done in a more selective way. From total fish production in 2016, of 171 million tons, about 88% was directly for human consumption, whereas in 1961, only 67% of the total fish production was to human consumption (FAO, 2018). Consequently, more of the world's fish production has been targeted to food and less to produce fishmeal and fish oil, with those products being supplied by exploratory fishing, which is showing a general decline in the past years, with marine fishing declining, continental (inland) fishing still growing but overall capture on a descend (Table 1).

Table 1 – Global fisheries and aquaculture production and utilization (in million tons) between 2011 and 2016

Category	2011	2016	Growth between 2011-2016 (%)
Production			
Capture			
Inland	10.7	11.6	7.8
Marine	81.5	79.3	-2.8
Total capture	92.2	90.9	-1.4
Aquaculture			
Inland	38.6	51.4	24.9
Marine	23.2	28.7	19.2
Total aquaculture	61.8	80.0	22.7
Total world fisheries and aquaculture	154.0	170.9	9.9
Utilization			
Human consumption	130.0	151.2	14.0
Non-food uses	24.0	19.7	-21.8
Population (billions)	7.0	7.4	5.4
Per capita apparent consumption (kg)	18.5	20.3	8.9

Source: Adapted from FAO (2018)

Aquaculture is the main source of fish for food in detriment of exploratory fishing. In the 1970s decade, 6% of the fish was from aquaculture, with the majority of it being from exploratory fishing and in 2006, half of the fish consumption was from aquaculture (FAO, 2009). In 2016, about 53% of the world's fish used for human consumption was from aquaculture production, with 80 million tons. Total capture has decreased by 1.4% between 2011 and 2016, a decrease also seen in the utilization for non-food uses of the products from fisheries and aquaculture (Table 1). Meanwhile, inland aquaculture showed an increase between those years of almost 25%, being the source of 51.4 million tons of fish (Table 1), or 64.2% of the global farmed fish production, while in 2000 inland aquaculture was responsible for only 57.9% (FAO, 2018).

Brazil has a big potential for aquaculture, due to its large territorial extension, wide coastline and huge freshwater availability, with 12% of the world's freshwater (MPA, 2014) and high fish species diversity (CEMIG/CETEC, 2000).

According to FAO (2018), Brazil's aquaculture production, in live weight equivalent, in 2016 was 581 thousand tons and the expectation is that in 2030 the country will produce 1097 thousand tons, with an 89% growth. Minas Gerais was the third state in tilapia production in 2017 (IBGE, 2017b), having surpassed Ceará and with its high hydric potential, the state can eventually outproduce Paraná and São Paulo in the coming years. Therefore, not only the country's fish production will increase, but it is safe to assume that tilapia production will be the force pulling Brazil's aquaculture production forward in the next decade and that states with large bodies of water, just like Minas Gerais, will be on the forefront of this growth.

2.2 Nile Tilapia (*Oreochromis niloticus*)

Nile tilapia (*Oreochromis niloticus*) is a fish natural from the African continent, Israel and Jordan, being found in the basins of the Nile, Niger and Chari rivers as well as lakes in the African Midwest (VERANI, 1980). Due to its wide distribution, adaptation and good acceptance of the consumer market, tilapias are produced all over the planet, emphasizing the need to increase even more the knowledge about this species (AZEVEDO et al., 2014).

It is a rustic species, of omnivorous alimentary habit, that may consume insects on its natural environment, with an optimal temperature between 26 and 28 ºC, adaptable to a wide range of feed and confinement production, being docile, with easy reproduction, high carcass yield and meat with good organoleptic characteristics, without thorns (SEBRAE, 2014; BOSCOLO et al., 2001).

Nowadays, it is the most disseminated and produced fish in the world's aquaculture and Brazil is the seventh largest producer in the world according to FAO (2014), but Brazilian data points out that Brazil already reached the world's fourth position in tilapia production (PEIXE BR, 2018). It is a recognized source of protein in developing countries, containing essential amino acids, essential fatty acids, iron, iodine, vitamin D and calcium (FAO, 2018; McCONNELL et al., 2000).

Nile tilapia (*Oreochromis niloticus*) was introduced in Brazil along with Wami tilapia (*Oreochromis hornorum*) in the 1970s decade by the National Department of Works Against Drought (DNOCS) with the goal of production of fingerlings for fish restocking of public water reservoirs in the Northeast region and the promotion of fish production (SHULTER; VIEIRA FILHO, 2017). In the 1980s decade, a huge effort was made by São Paulo and Minas Gerais' hydroelectric plants to produce massive quantities of tilapia fingerlings for restocking programs as well as to sell to rural producers, rapidly disseminating tilapia in the Northeast and Southeast regions of Brazil, and soon to the South of the country (KUBITZA, 2003). It was in this decade that tilapia production started to be less of an income supplement to small producers to be a commercial activity (FIGUEIREDO JÚNIOR; VALENTE JÚNIOR, 2008), but only in the 1990s that actual production started to be diffused in the country, especially due to sexual reversion technology (KUBITZA, 2003), the popularization of "fish and pay" type of business (FIGUEIREDO JÚNIOR; VALENTE JÚNIOR, 2008) and the National Water Resources Policy in 1997, which allowed multiple uses for hydroelectric reservoirs, one of which was the aquaculture (ROUBACH et al., 2015).

Tilapia was the fourth fish species most produced in the world in 2016, with 8% of the world's total finfish production (FAO, 2018). In 2017, tilapia was the most produced fish species in Brazil, representing over half of fish-farming production in the country, with 58,4% (IBGE, 2017b). If compared to the aquaculture production in 2017, tilapia production alone was about 36% of the aquaculture in the country (Table 2), solidifying its position as the most produced freshwater fish species in Brazil.

Table 2 – Tilapia production in kilograms, reais and in percentage of aquaculture in Brazil, the Southeast region and in the state of Minas Gerais in 2017.

	Tilapia production (kilograms)	Tilapia production (thousands of reais)	Percentage of tilapia production relative to aquaculture (%)
Brazil	283 249 263	1 579 028	35.9
Southeast region	76 436 060	431 068	79.2
Minas Gerais	28 963 177	173 938	80.9

Source: Adapted from IBGE (2017a)

Both in the Southeast region and in the state of Minas Gerais, tilapia production is responsible for about 80% of the aquaculture production and the revenue is as big. In the Southeast region, the sales of tilapia fish accounted for R$ 431 million, with R$ 173 million coming from Minas Gerais state (Table 2). That means that the Southeast region is responsible for 27,3% of Brazil's tilapia revenue and Minas Gerais state is responsible for 11% of the country's tilapia sales.

Even though it is very productive and growing, the tilapia production sector still needs to overcome some barriers in order to achieve its full potential and yield higher profits.

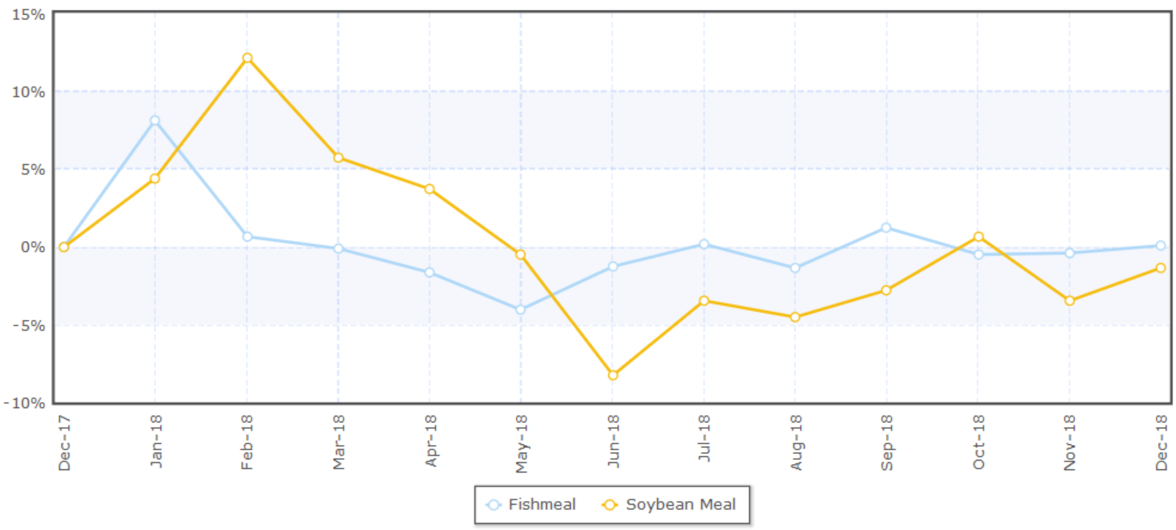
SCHULTER and VIEIRA FILHO (2017) highlight some critical factors that limit this growth: equipment availability for the activity, balanced feed specific not only to the species but for the region it is being produced, higher utilization of large fish cages and adoption of more intensity production systems. In this study we focused our work on a more sustainable balanced feed for Nile tilapia.

2.3 The soybean meal as aquaculture feed

Soybean meal use in aquaculture feed is widely spread due to its high protein content and amino acid profile (STOREBAKKEN; REFSTIE; RUYTER, 2000). Soy nutrients, however, have different effects on different fish species, ranging from decrease in growth and decreasing protein utilization (KROGDAHL; BAKKE-McKELLEP, 2001), intestine inflammation (KROGDALH et al., 2000) and even enteritis (MAHMOUD; KILANY; DESSOUKI, 2014). Therefore, new studies are focused on its substitution in fish diets.

The fishmeal rate of change in December 2018 was 0.06%, while the soybean meal rate of change in the same month was -1.41% (IndexMundi, 2019), as demonstrated in Figure 1, indicating that the price for fishmeal slightly increased, while it decreased for soybean meal in that same time frame. According to IndexMundi (2019), the fishmeal price in December 2018 was US$ 1480,06/metric ton while the soybean meal price was US$ 361,02/metric ton. In Brazil, however, in the past year soybean price increased in average 10.6% in the 16 states that produce this grain (CONAB, 2019).

Figure 1 – Monthly rate of change comparison between fishmeal price and soybean meal price between 2017 and 2018.



Source: IndexMundi (2019)

While the substitution of fishmeal by soybean meal is interesting economically and plant meals are the most common replacement for fishmeal, it is important to consider that plant-based diets can impair immune function and increase the susceptibility of infectious diseases to the animals because of the health problems caused by protein and amino acid deficiencies (OLIVA-TELES, 2012). As for farmed fish, the starch content and antinutritional compounds presents in plant meal diets could also decrease their health (FRANCIS; MAKKAR; BECKER, 2001) and soybean meal has antinutritional factors which makes it a limiting factor regarding soybean meal utilization in fish diets (HENDRICKS, 2002).

It is estimated that between 2005 and 2050 the population growth will increase global food demand by 100% (TILMAN et al., 2011), but agricultural production will not be able to increase at the same pace, reaching only an increase in 60% of its production (FAO, 2014). This means that while agricultural production will grow, it will not be enough to reach the demand. RAY et al. (2010) mentions that for some commodities, this increase in global production will not be enough to meet demands and by 2050 and soybean will face a shortage of approximately 55% (RAY et al., 2010). Agriculture will also face a number of challenges in the upcoming decades with the diminishing availability of farmland, climate change and the possibility of decline of water resources (PINOTTI et al., 2019).

Increasing crop production is a short-term solution for the problem (TOMBERLIN et al., 2015) because eventually demand will keep increasing and more area and resources will be necessary to produce more agricultural crops; consequently, there is a need for an alternative protein source to soybean meal in aquaculture feeds. Soybean meal has some antinutritional compounds and amino acid deficiencies (DUMAS et al., 2018; HENDRICKS, 2002), can cause mild to moderate enteritis in Nile tilapia, damaging the intestinal mucosa integrity, possibly affecting nutrient absorption and digestibility (MAHMOUD; KILANY; DESSOUKI, 2014; TRAN-NGOC et al., 2016).

Soybean meal is one of the predominant protein sources in commercial tilapia feed, averaging between 20-60% inclusion in diets (FAO, 2011; NG; ROMANO, 2013) and to produce a ton of soybean meal 2523 m^3 of water are necessary and 4190 m^3/ton to produce soybean oil (TSCHIRNER; KLOAS, 2017). In 2015, 5999 thousand tons of tilapia were produced, using 9178 thousand tons of feed (TSCHIRNER; KLOAS, 2017), thus, between 1120 and 3599 thousand tons of soybean meal were used in tilapia feeds in 2015 and between approximately 4.63-13.89 trillion litters of water were used in 2015 just to produce soybean meal to feed tilapia. The aquaculture is facing pressure to be more sustainable (PEREIRA et al., 2012), therefore, new studies are focused on finding sustainable and nutritious alternatives such as other agricultural crops, animal by-products, microorganisms and insects (DUMAS et al., 2018) for aquafeed.

2.4 Insects as protein sources for aquaculture

Insects are interesting protein, lipids, vitamins and mineral sources; however, their content will differ according to the insect's stage (NOGALES-MÉRIDA et al., 2018), taxon (BARROSO et al., 2014) and their diet (HENRY et al., 2015). They are under-appreciated as ingredients in human food and animal feed in most of the world. More than 1900 insect species are considered edible and nourish about two billion people, but the higher consumption occurs in Asia and Africa. This is the case because in many of the western countries, entomophagy is seen as a primitive action, which could only occur in the absence of any other food source. Therefore, research about edible insects have been neglected. However, the rise in the price of fishmeal and soybean meal commodities, along with the aquaculture growth creates a new need for research of insects in feed fish, and animal feed in general (VAN HUIS et al., 2013).

The interest on using insects as alternative protein source in aquaculture feed has been growing in the recent years (STAMER, 2015; FAO, 2013), especially because of their protein quantity and quality in several insect species (SANCHÉZ-MUROS; BARROS; MANZANO-AUGLIARO, 2014). And with the increase of the most common protein source prices (FAO, 2013; IndexMundi, 2019; CONAB, 2019), insect meal from mass-scale productions could become competitive in the future (DREW et al., 2014).

Insect production is seen as advantageous by FAO (2011) because they require minimum space and resources for production, do not compete directly with humans for food, their demand is greater than production and production rate and yield are high, creates money influx in a short period of time, insects are nutritious and part of human food for hundreds of years, are effective in converting food to protein, easy to transport and generally easy to breed, as well as do not require skilled labor (FAO, 2011). Omnivores species (such as Nile tilapia), pigs and poultry are suited to use insect and insect materials in their feed and convert them into high-quality food animal protein (PINOTTI et al., 2019). All these factors show the potential of raising and utilizing insects to animal feed. Aligning the production with low environmental impact, short life cycle and good acceptance by different fish species and even birds and reptiles (RUMPOLD; SCHLUTER, 2013), this is a niche market that can and should be explored in the upcoming years since the consumer market for animal derived proteins grows, and insects are being considered as alternative sources of proteins (VAN DER FELS-KLERX et al., 2016).

Zophobas morio belongs to the Coleoptera order and Tenebrionidae family. Most species from the Coleoptera order feed on carrion and excrements, can be predators or parasites and might infest stored animal and/or plant origin products (COSTA; IDE, 2006). This order has more than 350 thousand species, therefore corresponding to 40% of all insects and 30% of animals (LAWRENCE; BRITTON, 1991). Beetles from this order are variable in color and size, but usually have a uniform back, brown or cinereous color, the exoskeleton is relatively thick and hard, frosted or shiny, but almost always without hairiness (COSTA LIMA, 1955).

Its larvae are usually elongated, subcylindrical or somewhat depressed, with the tegument strongly sclerosed, glabrous and shiny and with short legs; *Zophobas morio* larvae (Figure 2) has a thick, leathery light brown exoskeleton, with dark rings along the body that are more prominent in the encephalic and caudal portions; they hatch measuring up to 2,5 millimeters in length and can reach up to six centimeters before developing to the pupa stage, usually growing between 2 to 4 centimeters at the time of the harvest (COSTA LIMA, 1955; FERREIRA et al., 2010; VAN HUIS; TOMBERLIN, 2017).

Figure 2 – *Zophobas morio* larvae.

Source: VAN HUIS; TOMBERLIN (2017)

Zophobas has been widely used in captive animals feed, as well as birds and fish, mainly in insectivorous species due to its easy production and nutritional value of its larvae (EBELING, 1975; SCHULTE, 1996). *Zophobas morio*, also known as superworm meal, could be used as partial replacement for fishmeal in farmed tilapia (JABIR; JABIR; VIKINESWARY, 2012), however, its utilization as a substitute for soybean meal and soybean oil has not been discussed in any research as far as our group is aware.

Table 3 summarizes the proximate composition of *Zophobas morio* both for its larvae and adult forms: dry matter varies between 38-95%, crude protein varies between 40-68%; crude fat averages between 14-40%; neutral detergent fiber (NDF), which is described by BAKER, FITZPATRICK and DIERENFELD (1998) as well as by OONINCX and DIERENFELD (2011) as chitin, varies between 13-50%; fiber varies between 0.4-32%; and ash averages between 2.7-6.2%. From Table 3 it is possible to observe that the *Zophobas morio* composition greatly varies, depending on the stage and, very possibly, on its feed, which was previously mentioned (NOGALES-MÉRIDA et al., 2018; BARROSO et al., 2014; HENRY et al., 2015).

Table 3 – Proximate composition of *Zophobas morio* in its larvae and adult forms.

	DM (%)	**CP (%)**	**CF (%)**	**NDF (%)**	**Fiber (%)**	**Ash (%)**	**References**
***Zophobas morio* - superworm meal (larvae)**	43 ± 1.4	40.8 ± 2.3	-	13.0 ± 6.8	0.4 ± 0.1	3.5 ± 0.6	BAKER; FITZPATRICK; DIERENFELD, 1998
***Zophobas morio* – superworm meal (larvae)**	92.49	47.42	40.01	-	-	3.54	JABIR; JABIR; VIKINESWARY, 2012.
***Zophobas morio* – superworm meal (larvae)**	94.57	49.91	33.05	-	-	2.77	FONTES, 2018.
***Zophobas morio* - superworm beetle (adult)**	38.21 ± 1.61	68.05 ± 0.62	14.25 ± 1.15	50.14 ± 0.93	32.06 ± 1.55	6.16 ± 1.85	OONINCX; DIERENFELD, 2011

DM = dry matter; CP = crude protein; CF = crude fat; NDF = neutral detergent fiber.

The amino acid profile for most insect species tested for aquaculture show a good correlation with fish requirements. While insects from Diptera order have their amino acid profile in more resemblance with fishmeal, those from Coleoptera and Orthoptera are similar to soybean meal, however with some deficiencies in lysine or methionine (BARROSO et al., 2014). In the case of the insects most used for fish diets will reach the requirement level for fish diets and some species, as *Zophobas morio*, the amino acid profile will even exceed the nutritional requirements (HENRY et al., 2015).

2.5 Digestibility Assays

One of the most important points when formulating diets for fish is to determine whether those fish can, in fact, digest the different ingredients (PEREIRA et al., 2012). Glencross, Booth and Allan (2007) define ingredient digestibility as "the measurement of the proportion of energy and nutrients, which an animal can obtain from a particular ingredient through its digestive and absorptive processes”. Digestibility assays are used when trying to determine ingredients potential nutritive value (HAJEN et al., 1993) and their potential for inclusion on fish diets (CHO, 1987). This need to understand ingredient utilization and its effects on animal digestibility led to the creation of methods to determine nutrient absorption, one of those being the apparent nutrient digestibility (VANDENBERG; DE LA NOÜE, 2001).

The apparent nutrient digestibility can be obtained by the direct or the indirect method (BOSCOLO; HAYASHI; MEURER, 2002). The direct method consists in total quantification of ingested feed and total feces collection, which is made difficult by the aquatic environment (MORALES; CARDENETE; SANZ, 1999) since the nutrients and organic matter can lixiviate from the feces into the water before collection (ALLAN et al., 1999), therefore, the direct method is not recommended for aquatic species, and in turn for those species, the indirect feces collection is indicated. The indirect method consists in the incorporation of a marker in the feed and then its determination in both the feed and feces to calculate the apparent nutrient digestibility (MAYNARD; LOOSLI, 1969).

The chromic oxide (Cr_2O_3) is the most used external marker in aquatic animals' assays (VANDENBERG; DE LA NOÜE, 2001), however some other markers are also available such as titanium oxide or barium and lithium carbonate (NRC, 2011). The diet's apparent digestibility coefficient can be estimated by the equation 1, proposed by Nose (1960) and the nutrient's apparent digestibility coefficient can be estimated by the equation 2, proposed by Cho, Cowey and Watanabe al. (1985).

$$Equation\ 1\ (Nose, 1960)$$

$$ADCd\ (\%) = 100 - \left(100\ \times \frac{\%\ marker\ in\ the\ diet}{\%\ marker\ in\ the\ feces} \times \frac{\%\ nutrient\ in\ the\ feces}{\%\ nutrient\ in\ the\ diet}\right)$$

Where: ADCd = Apparent digestibility coefficient in the diet.

$$Equation\ 2\ (Cho, Cowey\ and\ Watanabe, 1985)$$

$$ADCing\ (\%) = \left(\frac{100}{X}\right) \times (\left(\frac{test}{100}\right) - \left(\frac{Y}{100}\right) \times \left(\frac{basal}{100}\right))$$

Where: ADCing = Apparent digestibility coefficient of the ingredient's nutrient.

X = Percentage of ingredient's inclusion in the test diet;

Y = Percentage of the control diet;

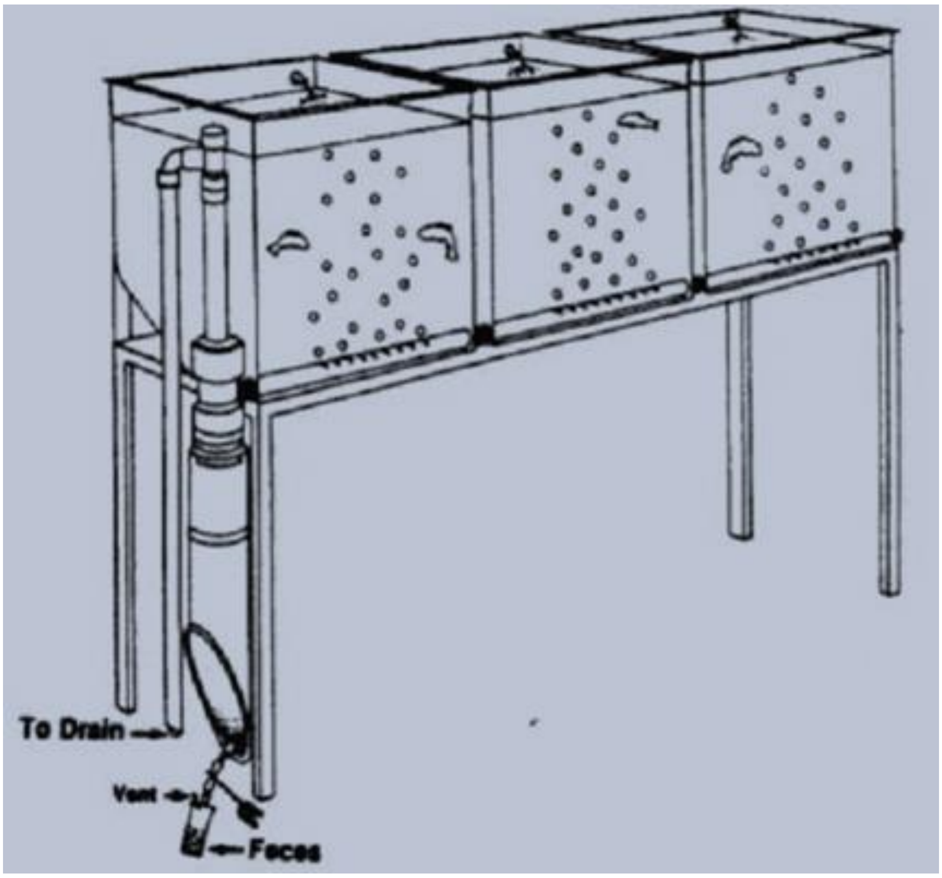
Test = Nutrient apparent digestibility coefficient in the test diet;

Basal = Nutrient apparent digestibility coefficient in the basal diet.

Feces collections, in the indirect method, can be done by either collecting decanted feces in the tank (SMITH; LOVELL, 1971), from the effluent water via filtration (OGINO; KAKINO; CHEN, 1973) or decantation column (CHO; SLINGER, 1979) and even, by continuous effluent filtration and feces removal (CHOUBERT; DE LA NOÜE; LUQUET, 1979).

To collect feces via a decantation column, as proposed by Cho and Slinger (1979), a system called the Guelph system (Figure 3) was proposed by Cho, Slinger and Bayley (1982). It consists in inclined aquarium with a pipe connected to the angled bottom of this aquarium and connected to another pipe to an acrylic sedimentation column. The base of this sedimentation column can be attached to an ice recipient in order to decrease the sample temperature and minimal fecal material degradation. Therefore, using this system, gravity will ensure that feces will flow to the pipe connected to the aquarium and it will reach the sedimentation column.

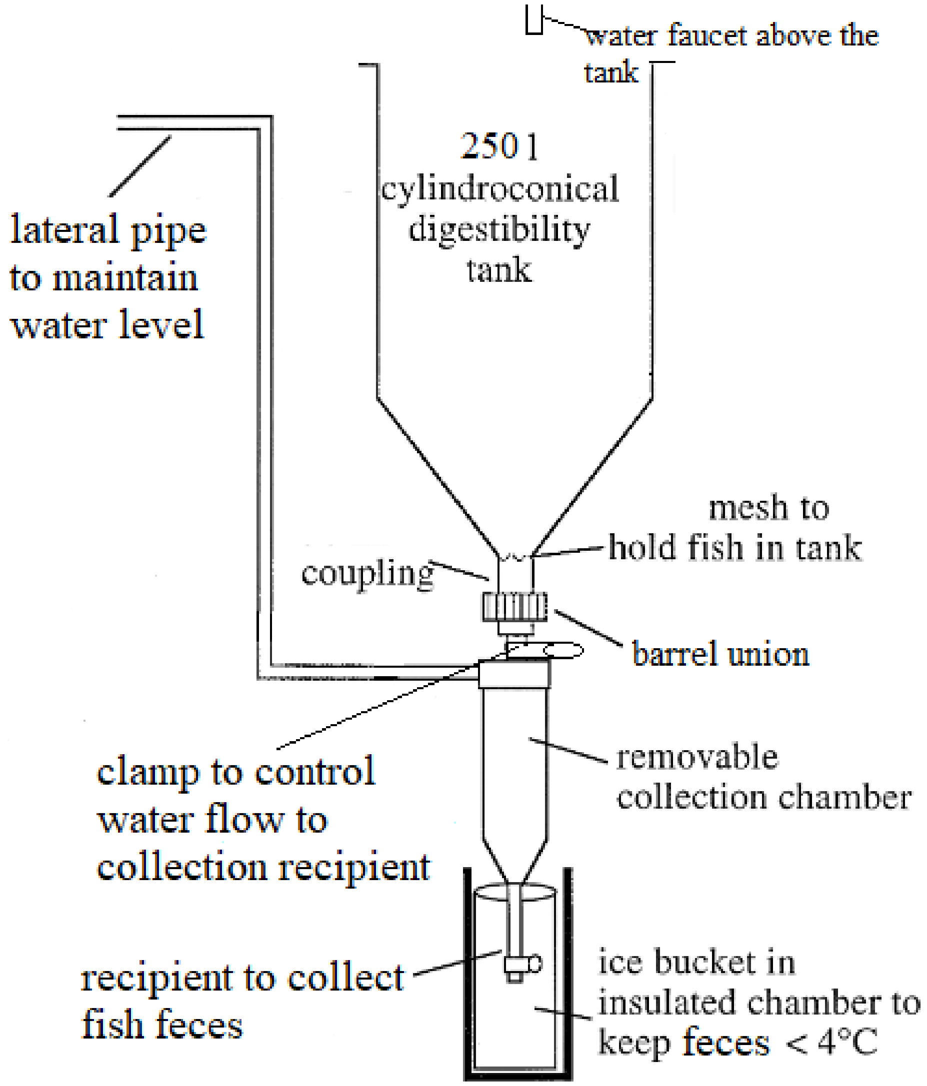
Figure 3 – Guelph system for fish feces collection as proposed by Cho, Slinger and Bayley (1982).



Source: CHO, SLINGER & BAYLEY (1982). In: Hancz & Varga (2018).

In Brazil the most used system is the adapted or modified Guelph system (Figure 4), which consists in a cylindroconical tank that has a lateral pipe connected to it that controls excess water in the digestibility tank, acting as a siphon (ABIMORAD; CARNEIRO, 2004). Attached to the bottom of the digestibility tank there is a recipient with a valve control, which allows it to be open after feeding the fish, allowing feces to decant, and to be closed to allow samples' collection (SAKOMURA; ROSTAGNO, 2007).

Figure 4 – Modified Guelph system



Source: Adapted from Allan et al. (1999)

While studying digestibility assays, there are two methods for ingredient inclusion on the test diets: a diet replacement method in which a test ingredient replaces part of the reference diet, and the ingredient replacement method, in which a test ingredient replaces a single reference ingredient that is often one at a moderate to high inclusion (GLENCROSS; BOOTH; ALLAN, 2007; AKSNES; HJERTNES; OPSTVEDT, 1996).

3 MATERIALS AND METHODS

The project was conducted at the Fish Culture Station at the Federal University of Lavras (UFLA) and at the Central Laboratory of Animal Research (LCPA/DZO), in Lavras, Minas Gerais. All experimental procedures were approved by the Ethical Commission of Animal Use (CEUA) of the Federal University of Lavras (UFLA), protocol number 041/18.

3.1 Handling and experimental diets

To compound the treatments, three diets were formulated with increased usage of insect meal (Table 4). Nutrient digestible values of the insect meal were acquired from previous research conducted in the same research group (to be published). The first treatment was a control diet, with 0% of *Zophobas morio* meal (Control), the second used 15% of *Zophobas morio* larvae meal (15 ZMM) and the third, had 30% of *Zophobas morio* larvae meal (30 ZMM) addition. The larvae were provided by the Entomoculture Laboratory from ICA/UFMG. The addition of insect meal was made in replacement to soybean meal and soybean oil, while fish meal levels were necessarily maintained the same in the three diets due to guarantee the reaching of the nutritional requirements of tilapia on this stage of development.

Table 4 – Composition of the experimental diets and calculated proximate composition (g kg $^{-1}$) with increasing levels of *Zophobas morio* meal inclusion for Nile Tilapia

	Control	**15 ZMM**	**30 ZMM**
Fish Meal	130.0	130.0	130.0
Zophobas morio Meal	-	150.0	300.0
Soybean Meal	195.0	97.5	-
Inert	58.0	50.0	40.0
Soybean Oil	87.0	42.5	-
Corn	174.0	174.0	174.0
Rice Bran	100.0	100.0	100.0
Corn Gluten	95.0	95.0	95.0
Wheat Bran	150.0	150.0	150.0
BHT[1]	0.5	0.5	0.5
Premix[2]	10.0	10.0	10.0
Chromic oxide[3]	0.5	0.5	0.5
Total	1000.0	1000.0	1000.0
Calculated Proximate Composition (dry matter basis)			
Dry Matter	889.5	857.1	849.5
Crude Protein	289.1	286.6	284.1
Gross Energy	4324.9	4448.6	4591.9
Crude Fiber	46.4	79.1	111.9
Total Lipids	130.6	133.7	138.8

Digestible Protein	264.2	262.8	261.3
Digestible Energy	3378.6	3373.4	3385.8
DE/DP3	12.8	12.8	12.9

[1]BHT (Butyl hydroxytoluene);
[2] Vitamin and Mineral Premix: vitamin A - 500.000 UI; vitamin D3 - 250.000 UI; vitamin E - 5.000 mg; vitamin K3 - 500 mg; vitamin B1 - 1.500 mg; vitamin B2 - 1.500 mg; vitamin B6 - 1.500 mg; vitamin B12 - 4.000 mg; folic acid - 500 mg; pantothenate Ca - 4.000 mg; vitamin C - 10.000 mg; biotin - 10 mg; Inositol - 1.000; nicotinamide - 7.000; choline - 10.000 mg; Co - 10 mg; Cu - 1.000 mg; Fe - 5.000 mg; I - 200 mg; Mn - 1500 mg; Se - 30 mg; Zn - 9.000 mg3. (Agromix LTDA, Sao Paulo, Brazil)
[3] DE/DP: digestible energy /digestible protein ratio

For that, 900 Nile Tilapia fingerlings (*Oreochromis niloticus*) with 3g average weight, were distributed randomly in 18 digestibility tanks with 50 fish per tank. A completely randomized design was adopted with three treatments and six replicates.

The digestibility assay was conducted in a water recirculation system with fiberglass tanks of 250L capacity, adapted to a modified Guelph system (Figure 4) with controlled water temperature (28ºC), mechanic filter and UV lamps, at the Fish Culture Station at the Federal University of Lavras - Animal Science Department.

The fish were fed twice a day (at 8:00h and at 14:00h), until they reached satiety. Every day the tanks were cleaned after the fish were fed at 14:00h. Then, in the water exit of each tank, tubes were fixed for the feces collection. Daily, at 8:00h the tubes were withdrawn, the feces were dried in a forced circulation heater at 60ºC for 36 hours and stored in refrigeration. The feces were collected for 15 days.

To determine the nutrients' apparent digestibility coefficient (ADC), the indirect methodology proposed by Cho and Kaushik (1990) was applied, using 0,5% chromic oxide (Cr_2O_3) as indicator in the final mixture of the diet ingredients. After that, the diets were pelletized and dried in a forced circulation heater at 60ºC for 24 hours. The dry mater (DM) ADC, as well as the crude protein (CP), ether extract (EE), ash (A) and crude energy (CE) ADC from feed were calculated according to Nose (1960) and Cho, Cowey and Watanabe (1985).

3.2 Centesimal composition

The bromatological analysis were made in the experimental diets and feces at the Central Animal Research Laboratory at DZO/UFLA. The dry matter, ash and crude protein were made according to the Association of Official Analytical Chemists (AOAC, 2012). First, diet and feces were macerated and homogenized to dry matter, ash, ethereal extract and crude protein; dry matter was determined after the samples were dried at 105ºC for 12 hours (AOAC, 2012).

Then the ether extract was determined according to the Folch method (1957), in which 200 mg of dried samples were added to 4 mL of chloroform: methanol solution (2:1), vortexed for 3 minutes and centrifuged for 20 minutes at 4000 rpm; the precipitate was discarded and 1 mL of water was added to the solution, homogenized for 40 seconds and centrifuged for 10 minutes at 3000 rpm; then the superior part was discarded and added 0,5 mL of a cleaning solution (3:48:47 chloroform: methanol: water) twice to eliminate residues; then 200 µL of methanol was added and left to dry; after 12 hours in the heater the samples were weighted and the difference was calculated (FOLCH, 1957)

The crude protein (N x 6.25) was determined by the Kjeldahl method, in which feed and feces samples were digested with copper sulphate, potassium sulphate and concentrated sulfuric acid and distilled with a Micro Kjeldahl appliance, where they were collected in in boric acid solution and mixed indicator (methyl red + bromocresol green) to then be titrated with 0.02 N hydrochloric acid (AOAC, 2012). Corrected crude protein was determined by using the same results from the Kjeldahl method, however, the nitrogen-to-protein correction factor was 4.76 (Kp = 4.76) instead of the usual 6.25, as described by JANSSEN et al. (2017), in which nonprotein nitrogen are not considered.

Ash determination was made by incineration on a gas stove and then placed in a muffle furnace at 600ºC for 5 hours (AOAC, 2012). The crude energy was measured with an adiabatic calorimeter pump (IKA C7000, Staufen, Germany) at the Biomass Energy Laboratory of the Forest Engineering Department of the Federal University of Lavras. The chromium oxide concentration was analyzed according to Bremer Neto et al. (2005) at the Department of Soil Science in the Federal University of Lavras.

3.3 Statistical analysis

The data was analyzed using a one-way ANOVA, using the R version 3.4.0 (2017, Vienna, Austria). Data was tested for normality with a Shapiro-Wilk test and to detect significative differences between the digestibility coefficient, the averages were compared using the Tukey test set at 5% of significance.

4 RESULTS

The proximate analysis of experimental diets is described on Table 5 and the proximate analysis of the feces collected using the modified Guelph system are described on Table 6.

Table 5 – Proximate analysis of experimental diets

	Control	15 ZMM	30 ZMM
Dry matter (%)	90.16	90.47	89.84
Crude protein (%)	26.99	30.66	33.52
Corrected crude protein (%) [1]	20.55	23.35	25.53
Ether extract (%)	9.80	10.27	11.23
Ash (%)	15.03	12.08	11.97
Gross energy (kcal/kg)	4324.9	4448.6	4591.9

[1] Corrected crude protein was calculated by applying a nitrogen-to-protein conversion factor Kp = 4.76, as described by Janssen et al. (2017).

The ash content in the diets decreased as insect inclusion increased (Table 6) and this can be explained by the decrease in inert addition in the diets, as shown on Table 4.

Table 6 – Proximate analysis of feces collected using the modified Guelph system

	Control	15 ZMM	30 ZMM
Dry matter (%)	91.78 ± 1.17	91.48 ± 1.50	90.55 ± 1.04
Crude protein (%)	11.59 ± 2.87	12.93 ± 1.24	14.11 ± 2.61
Corrected crude protein (%) [1]	8.83 ± 2.19	9.85 ± 0.95	10.74 ± 1.99
Ether extract (%)	3.67 ± 1.27	3.74 ± 1.46	4.29 ± 1.02
Ash (%)	27.36 ± 2.31	24.82 ± 1.39	23.08 ± 2.05
Gross energy (kcal/kg)	3351.83 ± 185.69	3269.00 ± 141.77	3231.33 ± 140.01

[1] Corrected crude protein was calculated by applying a nitrogen-to-protein conversion factor of Kp = 4.76 as described by Janssen et al. (2017).

As for the apparent digestibility coefficient of the experimental diets, the results are shown on Table 7. The apparent digestibility coefficients varied between 63-83% for dry matter, 84-93% for standard crude protein and corrected crude protein, 86-94% for ether extract, 34-66% for ash and 72-87% for gross energy. In all parameters, inclusion of 15% *Zophobas morio* meal in substitution of soybean meal resulted in the higher apparent digestibility coefficient compared to the control diet; 30% inclusion was similar to the control diet. The apparent digestibility coefficients of the diets with insect meal varied between 83-69% for dry matter, 93-87% for crude protein and corrected crude protein, 94-88% for ether extract, 41-66% for ash and 87-78% for gross energy, however, there were no significant differences between 15% or 30% insect meal inclusion in the diets.

Table 7 – Apparent digestibility coefficient of the experimental diets

	Control	15 ZMM	30 ZMM
Dry matter (%)	63.83 ± 6.02 b	83.68 ± 2.86 a	69.56 ± 5.66 ab
Crude protein (%)	84.39 ± 4.78 b	93.13 ± 1.35 a	87.26 ± 2.70 ab
Corrected crude protein (%) [1]	84.39 ± 4.78 b	93.13 ± 1.35 a	87.26 ± 2.70 ab
Ether extract (%)	86.68 ± 4.34 b	94.26 ± 1.63 a	88.25 ± 3.69 ab

Ash (%)	34.32 ± 11.23 b	66.35 ± 6.55 a	41.50 ± 11.11 ab
Gross energy (kcal/kg)	72.06 ± 4.30 b	87.95 ± 1.95 a	78.55 ± 4.16 ab

[1] Corrected crude protein was calculated by applying a nitrogen-to-protein conversion factor of Kp = 4.76, as described by Janssen et al. (2017). Averages with different letters in the same line represent significative difference between the treatments; Tukey test ($P < 0.05$).

5 DISCUSSION

When comparing the control treatment with 15 ZMM and 30 ZMM, it is possible to observe that all nutrients' apparent digestibility coefficient increased with the use of 15% *Zophobas morio* meal in substitution for soybean meal, while the inclusion of 30% insect meal was similar to the control diet. This might be due to changes on dietary fiber content and profile. Even though soybean and insect meal share the presence of a non-soluble polysaccharide as a common characteristic, soybean meal has approximately 10.5% ADF and 11.3% NDF according to Dilger et al (2004), known be a cause of enteritis in Nile tilapia, affecting the intestinal mucosa integrity, thus, possibly affecting nutrient digestibility (MAHMOUD; KILANY; DESSOUKI, 2014; TRAN-NGOC et al., 2016).

In the literature, there are few studies evaluating the potential use of *Zophobas morio* in fish diets. In the first, Jabir, Jabir and Vikinesawary (2012) replaced fishmeal by *Zophobas morio* meal in a 0, 25, 50, 75 or 100% of substitution (with ZM levels of 0, 7.5, 15, 22.5 and 30% total in the diet) for *Oreochromis niloticus* fingerlings. They found that diets with 25 or 50% of fishmeal replacement promoted an increased growth performance and was suitable for feed utilization.

In another study, Jabir, Razak and Vikineswary (2012) evaluated the apparent digestibility of superworm meal in juvenile red tilapia. In this case, fish were fed a mix with 70% of a reference diet and 30% of the test ingredient, which was either fishmeal (FM) or *Zophobas morio* meal (ZM). The apparent digestibility coefficient (ADC) for dry matter (DM), crude protein (CP) and crude fat (CF) were, respectively, 63.96%, 77.48% and 91.51% for the diet with fishmeal and 43.85%, 50.53% and 69.0% for the diet with ZM inclusion. The authors discuss that even though the ADCs were lower for the diets containing *Zophobas morio* compared to fishmeal, the ZM diet was still suitable for fish feed, as it fulfilled the requirements determined by FAO for fish nutrition (JABIR; RAZAK; VIKINESWARY, 2012).

In contrast, the apparent digestibility coefficients in our study were higher than the ones found by Jabir, Razak and Vikineswary (2012), even if compared with their fishmeal diet. This could be because their experimental diet was composed of 70% of a balanced diet and 30% of the test ingredient while ours incorporated the test ingredient in the diet formulation, and consequently, our ingredients were more well balanced and possibly, the nutrients were more available to the fish; digestibility methodology differences could also have an impact on the results. Fountoulaki et al. (2005) emphasize that digestive enzyme activities and nutrient digestibility can be affected by diet composition, which seems to be the case when comparing our results with the ones found by Jabir, Razak and Vikineswary (2012).

However, studies evaluating soybean meal substitution by *Zophobas morio* are scarce in fish nutrition, especially for Nile tilapia, *Oreochromis niloticus*. Using black soldier fly (BSF), *Hermetia illucens*, Dietz and Liebert (2018) evaluated insect meal as substitute for soybean protein in tilapia feeds. They used 25, 50 or 100% defatted BSF substituting soybean protein and up to 50% replacement (up to 18.5% BSF total in the diet) improved the protein quality of the tilapia feeds (DIETZ; LIEBERT, 2018). The insect meal inclusion in the diet for Jabir, Jabir and Vikineswary (2012) was up to 15% inclusion of *Zophobas morio* and for Dietz and Liebert (2018) was up to 18.5% inclusion of *Hermetia illucens*, whereas in our case, up to 30% *Zophobas morio* yielded the best results for Nile tilapia, without a depreciation in the digestibility coefficients.

A few studies evaluating the digestibility of *Zophobas morio* on other non-ruminant species diets were conducted recently. Ji et al. (2016) studied the inclusion on 5% ZM instead of 5% plasma protein powder in diets for early-weaned piglets. The basal diet had an ileal apparent digestibility of 87.58% for crude protein and 84.79% for dry matter for 1-28 days piglets, while with 5% inclusion of ZM the ileal apparent digestibility was 87.56% and 80.48%, respectively for crude protein and dry matter. For piglets between 29-56 days, basal diet had 79.60% ileal apparent digestibility for crude protein and 74.76% ileal apparent digestibility for dry matter and 5% ZM had, respectively, 79.12% and 74.09% (JI et al., 2016); therefore, the inclusion of ZM did not affect apparent ileal digestibility for piglets, however, digestibility decreased with age for both basal and ZM diets.

Our results for apparent digestibility coefficient of *Zophobas morio* in diets for *O. niloticus* were numerically higher for crude protein, no matter at what level of substitution, if compared with ZM substitution in piglets' diets (Ji et al., 2016). Apparent digestibility collection methods can have different results if comparing fecal apparent digestibility and ileal apparent digestibility, therefore, comparing our data with ileal apparent digestibility in piglets should be done with caution. However, the utilization of *Zophobas morio* in non-ruminant diets is interesting and seems to not to bring any damages on nutrient digestibility when compared to control/basal diets and ZM inclusion yields high digestibility coefficients.

6 CONCLUSIONS

The total substitution of soybean meal by *Zophobas morio* meal with inclusion up to 30% in diets for Nile tilapia fingerlings, *Oreochromis niloticus*, is beneficial regarding the improvements on dietary nutrient's digestibility without any depreciation of the apparent digestibility coefficient; however, a 15% inclusion of *Zophobas morio* meal yielded the higher apparent digestibility coefficients when compared with the control diet.

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