



UNIVERSITI PUTRA MALAYSIA

***CHARACTERIZATION AND UTILIZATION OF AZOLLA PINNATA WITH
DECANTER CAKE WASTE AS POULTRY FEED***

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APPROVAL SHEET

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ABSTRACT

Decanter cakes are the major wastes in crude palm oil industry which are currently disposed in the landfill or reuse as other applications. But on the other view, decanter cake is source of nutrients for producing bio-compound fertilizer and also suitable for animal food. In addition, *Azolla pinnata* comprises of significant high food value with a good proportion of protein, vitamins, and minerals. Therefore, the purpose of this project is to produce low-cost animal feed pellet by combination of *Azolla pinnata* (AP) with Decanter Cake (DC) at selected ratio. The proximate composition analysis of carbohydrate and protein in the pelletized AP and DC were analysed. In this study, 18 chicks at the age of 4 weeks were reared in six treatments, labelled control, T1 to T5, by substituting 0% (only maize), 100%, 95%, 90%, 85%, and 80% of *Azolla pinnata* protein (dry matter basis) respectively for 29 days. The specific growth rate of chicks were measured daily. There were not significantly varied between the chicks reared completely with maize and 95% substitution with *A. pinnata*. However, a significantly higher weight was gained for chicks reared in T5 than other treatments. The poorest performance was observed in chicks fed completely with *Azolla pinnata*, at control and T4. Based on the results, pellet made of ratio 20% of decanter cake and 80% of *Azolla* could be substituted with maize without significantly lowering their growth and product quality. As a conclusion, the poultry feed formulated of *Azolla pinnata* and decanter cake able to improve the weight of chicks. This feed pellet can be used as an alternative food source for poultry industry in Malaysia.

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LIST OF ABBREVIATIONS

AP	:	Azolla pinnata
BOD	:	Biochemical Oxygen Demand
CV	:	Coefficient of Variance
DC	:	Decanter Cake
d.w	:	Dry Wet
EFB	:	Empty Fruit Bunch
FA	:	Fatty Acid
FFB	:	Fresh Fruit Bunches
g	:	Gram
PKC	:	Palm Kernel Cake
PKE	:	Palm Kernel Effluent
PKS	:	Palm Kernel Shell
PPF	:	Palm Press Fibre
PODC	:	Palm Oil Decanter Cake
POME	:	Palm Oil Mill Effluent
RPD	:	Relative Protein Digestibility

CHAPTER 1

INTRODUCTION

1.1 Background

Fish and poultry are the major animal protein sources for human consumption and their feed conversion efficiencies are higher than those of other organisms. However, the success of rearing fish and poultry depends upon the feed given so the feed should be prepared based on the precise knowledge of their nutritional requirements because the optimum growth can be achieved in a given time. In addition, the balanced diet to be given to these animals should contain nutrients such as protein, carbohydrates, lipid, vitamins and minerals to achieve the energy requirements and also to ensure their healthy growth. Based on all of components of the formulated feed, protein plays an important role in the feed production and also it is as costly component. Besides protein, the animals also require other nutrients like carbohydrates and lipids and it is well known that supplements of carbohydrates or lipids have a sparing effect on dietary protein being used as energy source in higher animals.

Animal feed is any single or multiple materials whether processed, semi-processed and raw, which is intended to be fed directly to food producing animals including feedstuffs, ingredients, additives and supplements (Department of Standards Malaysia, 2005). Currently, the use of animal feed ingredients, including animal waste, rendered animal products, metals, antibiotics, and fats could result in higher levels of antibiotic resistant bacteria and dioxin like compounds in animals and resulting in animal based food products intended for human consumption (Gulland, 2013). Nowadays in Malaysia

commonly livestock feed have been produced from grain corn. Recently, feed was prepared in the form of pellets for the fish and in the form of mash for the broilers.

Agro-industrial by-product could be alternative as cheap and sustainable feed for livestock because limited feed resources are the most important constraint for animal production industries in tropical areas. Besides that, it has been reported that 60-70% of the production cost for livestock growers are from animal feed (Zahari & Wong, 2009) and at the same time, they are facing difficulties in complying with ceiling price of poultry meat set by the Malaysia government. To comply with the ceiling price of poultry meat, poultry breeder need to be efficient and productive in their operation by reducing the cost surrounding poultry meat production for example by finding ways to reduce feed cost (United Nations Economics and Social Commission for Asia and the Pacific ESCAP, 2014).

Global Agricultural Information Network (2013) has reported that the total overall import of corn into Malaysia are increasing from 4.0 million in 2017/18 to 4.1 million in 2019 and because of that the exportation of corn in US are likely increase to 320,000 tons due to positive acceptance of U.S corn among Malaysian feed millers. Besides that, they also have reported the wheat export to Malaysia from U.S also be increasing to 210,000 tons as demand for quality bread and bakery good. In another word, the demand for poultry product have risen from day to day due to popularity of chicken as main menu in various chain restaurants such as KFC, The Chicken Rice Shop and Ayamas.

Moreover, in poultry production the total expenses greatly influenced by feed price that can reach up to 70% of total cost. Feed component of total costs for broiler production increase from 51.8% in 2001 to 68.7% in 2008 at peak of food crisis (Donuhue and Cunningham, 2009)

Furthermore, Zahari and Wong (2009) have concluded that the utilization of local feed sources is highly dependent on supply of agro-industrial by product or crop residues from oil palm and rice industries. Meanwhile, in their studies the ex-farm production value of non-ruminant subsector was valued at RM 849 million. Besides, Zahari and Wong (2009) also asserted Malaysia was highly imported the animal feed especially for non-ruminant subsector (poultry and swine) and aquaculture. Commonly, maize and soya bean meal are the main imported energy and protein feed and statically the value of importation is approximately RM 2.5 billion per year.

Azolla pinnata are aquatic plant, free floating fern and floating aquatic macrophytes which belongs to family Azollaceae (Cherryl, et. al, 2014). It has been found as submerged, emerged (rooted) and free-floating species and has a potential valuable as feedstock for next generation biofuels because their ability to produce a large amount of biomass, impressive bioremediation rates, as well as cheap and easy maintenance and harvesting (Cui and Cheng,2015). Also, *A. pinnata* appears as a good source of protein and contains almost all essential amino acids that are superior to wheat bran, maize, etc. Generally, the crude protein content of that plant species is found in the range from 25% to 30% in dry matter basis at optimum growth conditions. In many years, raw and dried *Azolla pinnata* has been used as supplemental fish feed for their growth (Mousumi et al., 2018). Therefore, *A. pinnata* is suitable and has potential to be used as substitute to poultry feed due to its protein content which is comparable or higher than that of most other aquatic macrophytes.

In Malaysia, among palm oil by-product waste, palm oil decanter cake (PODC) is potential, available and cheap feed resource for animal feed (Wan Zahari et al., 2011). The palm oil decanter cake has also been used as alternative feed resource for ruminants such as goats (Gafar et al., 2013) and fish feed (Chang & Chuah, 2014). The study by

Chang and Chuah (2014) concluded that the high levels of crude fiber and marginally low content of crude protein in decanter cake of palm oil mill which make it unsuitable to completely replace the conventional fish feed. However, the in vitro protein digestibility result indicates that it is readily digested and may incorporate as part of the protein source in fish feed formulation. The proximate analysis of decanter cake such as crude lipid, crude protein, crude fiber, and ash contents based on dried weight were 9.7, 13.9, 17, and 8.7 % respectively. The fresh decanter cake showed approximately 23.5 and 54.9 % relative protein digestibility (RPD) using fish gut crude enzymes of Nile tilapia and catfish, respectively. The RPD obtained indicates that the decanter cake can be part of the protein source for both types of freshwater fish studied.

So far, most of the study used raw *Azolla pinnata* as supplemental diet together with the commercial animal feed. Therefore, it would be an excellent inexpensive animal feed if the combination of decanter cake and *Azolla pinnata* could be utilized as a commercial animal feed ingredient. Thus, the aim of this study was to examine the growth and feed utilization, of chicks fed with substituting different levels of decanter cake with dried *Azolla pinnata*.

1.2 Problem statement

As mentioned above, protein plays an important role in animal feed for the growth. However, the high cost of commercial feed is limiting the potential development of intensive poultry or livestock industry. The animal feed industry needs cheaper and readily available protein ingredients. Consequently, this problem can be solved by using the low-cost sources which is *Azolla pinnata* and decanter cake. *Azolla pinnata* has been used for centuries in agriculture field. Available literature indicates that *Azolla pinnata* is an economic and efficient feed supplement for different species of animals, containing substantial amounts of protein, amino acids, vitamins and minerals which significantly

reduce the cost of feeding. *A.pinnata* was found to be rich with protein at about 22% dry wet basis (d.w) and it is easily to be planted in water. Thus, *Azolla pinnata* can be a potential source of protein and nutrients for livestock and has a considerably high feeding value (Anita et al., 2016). Agro-industrial by-products such as palm oil wastes are also suitable to be used as animal feed because of their high fibre and carbon content, although they lack of proteins and vitamins for example palm kernel cake (PKC) and decanter cake (DC). This is because of the high crude fibre content which comprised of mainly beta-mannans and other nonstarch polysaccharides. Subsequently, PKC and DC has been used mainly in ruminant feed. In the last 10 years or so, much effort have been directed towards using PKC as feed for poultry. Its high crude fibre content limits its use in poultry ration to about 15%. Adding cellulase, mannanase and other cocktails of enzymes mixtures have apparently improved the digestibility. However, so far there is no conclusive evidence to support the feasibility of using DC for poultry. Nevertheless, DC still remains an option as a method of improving nutritive value. Studies are still ongoing on the poultry diet. The use of by-product waste in poultry diet by local feed millers is very limited and inclusion level is usually less than 5% so if the waste is used at the rate of 100% in poultry diet, it is estimated around RM 184 million in imports can be saved.

Since decanter cake is rich in fibre and *A.pinnata* has high protein content, both has possess certain amount of beneficial nutrients for animal growth. Therefore, this study will utilize the decanter cake and *Azolla pinnata* as the main ingredients in animal feed formulation. The dried *Azolla pinnata* and powder form of decanter cake will be formulated into pellet as to ensure the suitable applicability of the mixture to be used for poultry feed.

1.3 Research objectives

The aim of this research is to investigate the utilization of *Azolla pinnata* with decanter cake waste for livestock feed and improvise them from raw material to pellet feed. There are specific objective for this research:

1. To study the characteristic of *Azolla pinnata* and Decanter cake.
2. To produce animal feed pellet at the selected ratio of *Azolla pinnata* and Decanter Cake.
3. To evaluate the poultry growth at the selected ratio feed.

1.4 Scope of research

In order to achieve the listed objectives, this research study carried out with these limitations. The raw material analysed is palm oil mill by-product, specifically the decanter cake collected from a palm oil mill at Jengka, Pahang. The sample of *Azolla pinnata* was collected from Food and Processing Engineering Laboratory, UPM. Both of sample was main ingredient to produce pellet feed and the binder used was tapioca powder with water. The binder is used to ensure the pellet feed easy to be shaped and diameter need to be maintained within 2 mm to 3.5 mm. The pellet feed was produced based on selected ratio with requirement of animal feed prior tested to poultry chicks. The requirement of pellet feed produced have been analysed by using proximate analysis to determine the content of protein and carbohydrate. The pellet feed produced with selected ratio been fed to chicks and their weight were monitored daily.

CHAPTER 2

LITERATURE REVIEW

2.1 Livestock in Malaysia

The Malaysian livestock industry is an essential and basic sector of the agricultural division, giving profitable job and delivering valuable animal protein food for the population. The industry can be divided into the non-ruminant and the ruminant sub-areas (Loh,2001). For some types of livestock, including dairy cows, steers, poultry and swine, nutritious supplements are fused into concentrate diets, which for the most part guarantee that animals are getting the obliged nourishment and minerals (Lee, 1996). Besides that, Dahlan et al. (2013) emphasise that energy and nutrient are important supplements for animals especially ruminants like cattle. The most obvious way of increasing the voluntary intake of forage is to provide supplements in order to balance the nutrients absorbed by the cattle. Providing supplements with relatively high protein concentrations to enhance forage use and livestock performance (Perdok and Leng, 1990).

2.2 Ruminant and Non-Ruminant Animal

As have mentioned above the animal in the world has been divided to two which is non-ruminant and ruminant. In 2008 ex-farm production value of the non-ruminant sector in Malaysia was estimated to be around RM 9003 million while for ruminant subsector was valued at RM 849 million.

The non-ruminant livestock is animal that have a simple stomach structure with a single compartment which facilitates the normal digestion process where the ingested food is digested in single process. Non-ruminants is include most of the carnivores, omnivores, and some herbivores which contain a simple stomach structure and they do

not undergo regurgitation process as in ruminants. Mostly, non-ruminants can be categorized as chicken, duck, pig and eggs.

The ruminant livestock is different from another monogastrics such as swine and poultry because ruminants have a digestion system that designed to ferment feedstuffs and provide precursors for animal energy use. In other word, ruminants are hoofed mammals that have a unique digestive system that allows them use energy in better way from fibrous plant material than other herbivores. So, ruminant can be recognized as animal that have 4 compartment or easy meaning the animal that have 4 legs.

2.3 The Physiology of Azolla Plant

Azolla can be recognized as water fern that widely distributed in aquatic habitats like ponds, canals, and paddy fields in temperate and tropical climatic regions. This is because *Azolla* is a kind of weed that is easy to breed and grow, and it also only require a minimum depth of three inches of water and use organic fertilizer such as cow or goat dung, or NPK fertilizer (nitrogen, phosphorus and calcium) (Utusan Malaysia, 2018). Besides that, *Azolla* also known as mosquito fern, fairy moss and water fern. Recently, *Azolla pinnata* has also become increasing popular because of its biomass production and bioremediation potential (Miranda et al.,2014).

In addition, this fern consists of six species that which known within genus that grouped into two subgenera which is *Euazolla* and *Rhizosperma* (Pradesh et al., 1991). Besides, Pradesh et al. (1991) also has characterize *Azolla* is green and will becomes red for certain *Azolla* because temperature of water (Hussner, 2010). Utusan Malaysia (2018) also state that *Azolla* is recognize to have a unique shape, does not look like a normal fern

but it looks more like duckweed or several of moss and in Asia their species has been known as *Azolla pinnata*. Then, the shapes of *Azolla* plant are triangular or polygonal and float on the water surface individually or in mass (Ferentinos, et. al, 2002) and also the diameter size of *Azolla* will be in ranges from 1 cm to 2.5 cm (Pradesh et a., 1991).

They also known as a good source of protein because has a higher protein content than green forage crops and aquatic macrophytes (Cherryl, et. al., 2014). Besides that, it contains almost all essential amino acids, minerals such as iron, calcium, magnesium, potassium, phosphorus, manganese etc, apart from appreciable quantities of vitamin B12 (Pillai, et al., 2002). Based on its unique chemical composition *Azolla* become a new feedstock for bioenergy production. Together with their evolutionary symbiont, *A. Azollae*, *Azolla* representatives contain three major types of energy molecules, starch (approx. 6% d.w) and cellulose/ hemicellulose (up to 35% d.w) and lipids (8% d.w) which are found separately in known terrestrial feed stocks and microalgae (Miranda et al., 2016).

2.3.1 *Azolla* Species in the World

As mentioned above the *Azolla* plant consist six species in this world that grouped under two subgenera which is *Euazolla* and *Rhizosperma* (Pradesh et al., 1991). So, under *Euazolla* consists four species which are *Azolla filiculoides* *Azoll caroliniana* *Azolla Mexicana* and *Azolla microphylla* and they from temperate, sub-tropical and tropical region of North and South America (Pradesh et al., 1991). Then, for other two species is *Azolla nilotica* and *Azolla pinnata* that be under *Rhizosperma*. Hence, for *Azolla nilotica* it can be found in East Africa (Van Hove, 1989) and *Azolla pinnata* has two different varieties in distribution pattern which is *Azolla pinnata* var. *imbricate* (originates from

subtropical and tropical asia) and *Azolla pinnata* var. *pinnata* (occurs in Africa and known as Africa strain).

Figure 2.1 shows the picture of *Azolla filiculoides* and it is a small aquatic heterosphere fern that rarely larger than 25 mm because this genus is unique as it grows along with cyanobacterium heterosistus (blue-green algae). *Anabaena azollae* Strasburger (Nostocales: Nostocaceae), located in the dorsal leaf lobes (Hussner, 2010).



Figure 2. 1: *Azolla filiculoides* (Hussner, 2010).

Figure 2.2 shows *Azolla caroliniana* which is *Azolla Carolina*, or water velvet that native Azolla species in America, in eastern north America from southern Ontario to the south, and from the east to Wisconsin and Texas. Besides, in Central and South America from southern Mexico (Chiapas) South to north Argentina and Uruguay (Weakley, et al., 2005).



Figure 2. 2: *Azolla Caroliniana* (Weakle, e. al., 2005)

Figure 2.3 shown *Azolla Mexicana* that were found in western North America from southern British Columbia to Texas, and in the center of the United States. Its habitat is wetland species of backwater, cattle, wet ditches and stagnant ponds of Interior Douglas-fir and Interior Cedar Hemlock Biogeoklimatic Zones (Hemlock & Zones, 2015).

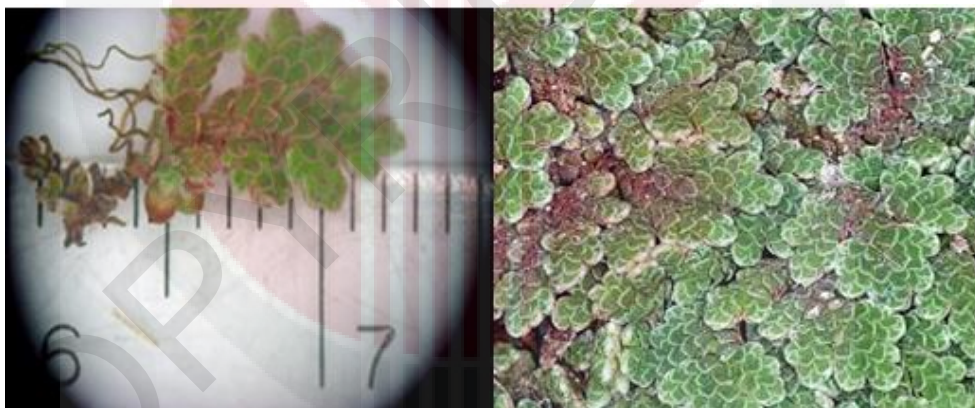


Figure 2. 3: *Azolla Mexicana* (Hemlock & Zones, 2015)

Figure 2.4 shown the *Azolla nilotica* and it is medium sized floating fern, which naturally occurs on the Nile and East and central Africa. It was assigned to the Salviniaceae family (Beentje, 2017).



Figure 2. 4: *Azolla pinnata nilotica* (Beentje, 2017)

Figure 2.5 below is *Azolla pinnata* species that quite common in the tropical regions of the world and the original ranges include Africa and Madagascar, India, Southeast Asia, China (Fern, 2015).



Figure 2. 5: *Azolla pinnata* in Malaysia (Pradesh et. al, 1991)

2.3.2 Types of *Azolla* plant in Malaysia

In Malaysia region, *Azolla* found to be under species of Rhizosperma which is *Azolla pinnata* var. *imbricate* and commonly called as *Azolla pinnata* because it originated from subtropical and tropical East Asia (Pradesh et al., 1991). Besides that, this species fairly common in tropical regions of the world and the native range includes Africa and

Madagascar, India, southeast Asia, China (Fern, 2015), Japan, Malaysia and Australia (Blyxa & Beentje, 2017).

Azolla pinnata needs water as a fundamental to growth and multiply and it is important source for *Azolla pinnata* (Pradesh et al., 1991). Then, the pH of water that suitable for *Azolla pinnata* is range at pH 4.5 to 7.0 for optimum growth and reported that the best pH for them is around 5.5 (Tung & Shen, 1981). Hence, the water needs to sprinkle with soil (Cherryl et. al., 2014) and the pH of soil should be in range 6 to 7 to support the best growth (Barham et al., 1997). Moreover, *Azolla pinnata* also can fully grow in partial shade 50 to 100 percent of sunlight while decrease quickly under heave shade of sunlight (Ferentinos, Smith & Valenzuela, 2002). Generally, *Azolla* needs 25 to 50 percent of sunlight for normal growth and biomass production will decrease at lower intensity of light than 1500 lux (Pradesh et al., 1991).

Composition Analysis	<i>Azolla pinnata</i> (%)	Corn (%)
Wet content (dry basis)	10.30	7.20
Protein	22.40	11.70
Fiber	21.9	1.7
Fat	3.2	3.68
Ash	24.20	2.07
Calcium	0.2	17.9
Phosphorus	0.8	0.8

Table 2. 1: Chemical Composition for *Azolla pinnata* and Corn (MEGA AGRO, Utusan Malaysia, 2018)

2.4 Palm Oil Waste

In Malaysia the oil palm sector known as one of major industries which since 1970, Malaysia has been the largest producer and exporter of palm oil products in the world. Oil palm industry in Malaysia has producing over 83 million dry tonnes of solid biomass annually and expected on 2020 it will increasing to between 85 and 110 million dry tonnes (Maniam et a., 2013). So, in the oil palm industry their co-product are obtained from two sources which namely from residues in the plantations (field residues) and from palm oil milling.

The best alternative for guaranteeing energy future is sources from biomass which is a promising sources of renewable energy that contributes to energy need. By- product that produce from oil palm include palm kernel cake (PKC), decanter cake (DC), palm oil mill effluent (POME), empty fruit bunch (EFB) and palm press fibre (PPF).

2.4.1 Decanter cake (DC)

In addition, there is other biomass production from oil palm which is decanter cake (DC). Decanter cake is a solid waste that had produced when the crude palm oil is centrifuged for purification where the supernatant is the purer palm oil and their sediment become decanter cake. The decanter cake is derived from the palm oil mill sludge separation and the material that remains after decanting the palm oil effluent. Most of the palm oil mills were currently disposed the decanter cake by incineration, inclusion in animal feeds, and land filling method or concrete manufacturing. Oil palm decanter cake is a brown-blackish paste (figure 3.4) obtained in the extraction phase of processing for palm oil (Seephueak et al, 2011). Most of the solid in the final effluent is decanted in a decanter and pressed to a filter press before being dried in a rotary dryer (Devendra et al.,

1981). Furthermore, Haron and Mohammed (2008) reported that a mill 90 t hr^{-1} FFB processing capacity will produce about 160-200 tonnes of DC per hour. Afdal et al., (2012) reported that decanter cake consists of 12.63% of crude protein, 7.12% ether extract, 25.79% crude fibre, 0.03% calcium and 0.003% phosphorus respectively. Then, the fatty acids (FA) components of DC are usually palmitic, oleic, linoleic, stearic and myristic acids (Afdal et al., 2012). Table 2.1 indicates the various palm wastes produced in the mill and different uses.



No.	Types of palm waste residue	Uses
1.	Fronds, trunks and leaves	<ul style="list-style-type: none"> • Used as mulching materials in the fields which helps in moisture retention. • Roofing material and processed as furniture.
2.	Empty fruit bunch (EFB)	<ul style="list-style-type: none"> • Generating steam for the mills and ash residues used as fertilizer (Lim, 2000) • As raw material for products such as panelling, composites, fine chemicals, pulp and paper as well as compost and bio-fertilizer (RamLi et al., 2001) • Main substrate for the cultivation of (oyster mushroom (Tabi et al., 2008)
3.	Palm press fibre (PPF)	<ul style="list-style-type: none"> • Substrate for animal feed in addition to soymeal, fishmeal. Potting material for ornamental plants to improve foliar growth (Yusoff, 2004) • Used for making fibre boards (RamLi et al., 2002) • Polymeric composites for building materials referred to as AGROLUMBER for products like wall panels, sub-floors, doors and furniture parts. (MPOB, 2009)

4.	Decanter cake (DC)	<ul style="list-style-type: none"> • Used in combination with inorganic fertilizer to improve soil quality on palm plantations (Haron and Mohammed, 2008) • Utilization of oil palm decanter cake for cellulose and polyoses production (Razak et al., 2012) • Feed for goats (Gafar et al., 2013) • Production of natural polymer composite (Adam et al., 2014)
5.	Palm kernel cake (PKC)	<ul style="list-style-type: none"> • Suitable as feedstock because it has 48% carbohydrate and 19% protein (Kolade et al., 2005)
6.	Palm kernel shells (PKS)	<ul style="list-style-type: none"> • Used mainly for fuel (Paepatung et al., 2006) • Converted into activated carbon for water purification purposes (Ortiz et al., 1992)
7.	POME	<ul style="list-style-type: none"> • Mainly used for Irrigation purposes • Carotenes are extracted from POME by pharmaceutical industries (Wood and Lim, 1989).

Table 2. 2: Various Palm Oil Mill Residues and their Utilization in Industries (Asha Embrandiri, 2016)

Typically palm oil mills generate 3.5% of oil palm decanter cake (OPDC) for each tonnes of fresh fruit bunches (FFB), this were reported from previous studies. Moreover, this waste become an attractive feedstock for biogas production because their biodegradable organic content is higher and rich of nutrient compositions. DC was containing water about 76% on wet basis, residual oil about 12% on dry basis and nutrients, cellulose, lignin and ash. Then, the oil adsorbed on DC is minor by-product of oil purification process with appreciable magnitude that could be a potential feedstock for production of biodiesel (methyl ester).

Besides, decanter cake are very difficult for storing, handling and burning because of high moisture content levels in the residues and when associated with the organics in the decanter cake, it also will cause air pollution and exert polluted gaseous like ammonia gaseous. Consequently, utilization of palm oil decanter cake will improve the environment because the disposal of sludge solid waste will increase the Biochemical Oxygen Demand (BOD) of the land. Bamikole and Babayemi (2008) reported that the sludge has good potential as feed and could be directly used in ruminant feeding as an energy source. Anwar et al., (2012) stated that DC is a valuable and potential by-product that can be utilized as an alternative energy and protein source for rearing goats.

According to Abubakr et al. (2014) DC can be an alternative as a cheap animal feed in order to overcome the problem of the scarcity of its sources. Earlier, the same group of researcher suggested that only small amount of DC needed in goats diet since high dietary level might affect the goats or small ruminant growth negatively (Abubakr et al., 2013). Study perform by Gafar et al. (2013), decanter cake can be an additional ingredients of kacang goats up to 30%. This believes to aids the animal feed crisis. While

Afdal et al. (2012) find out the fresh decanter cake is more suitable as an alternative feed especially for ruminant.

2.5 Livestock Pellet

Livestock industry in Malaysia shows a significant growth especially in poultry sector (United States Department of Agriculture (USDA), 2014). Mostly, now many of livestock animals will be feed with feed pellets, as feed pellets have higher nutrition density, more comprehensive nutrition, higher economic benefits so animals can digest, absorb and conserve better than raw material feed. Other than that, the pellet are easier to store and transport than traditional roughage (coarse fodder).

Besides, the usage of antibiotics as growth promoters to improve feed utilization and production relates to the accumulation of antibiotics in products from treated animals (Barton, 2000).). The purity and safety of the livestock meat is highly related to the variety of feed fed to the animals, since it is indirectly affecting human wellbeing (Nor, Abdullah & Rahman, 2011).

Abc Machinery (2018) identify generally livestock feed pellets have 4 kinds of classification of pellet which is pure forage (grass) feed pellets, complete diet feed pellets, concentrated feed pellets and premix feed pellets. There are several example for feed pellet capacity of livestock that state by Abc Machinery which is for cattle, cow, sheep need 0.1kg of concentrated feed pellet per day, and also pig needs 0.25kg concentrated feed pellet per days. Thus, 3kg concentrated feed pellets can transform to 1kg of meat for animals.

2.6 Pellet Process

Pelleting is a process that gives physical, nutritional and economic profits. In easy meaning pelleting is food that be compacting and forcing by any mechanical process that will become into any shape such as circle, cylindrical shape, irregular, square or oval. Meanwhile, California Pellet Mill.Co have define pelleting feed is “agglomerated feeds that formed by extruding individual ingredients or mixture by compacting and forcing through die openings by any mechanical process” in their journal Animal Feed Pelleting. Usually, pelletizing give better feeding result when compared to un-pelleted feed because in purpose of pelleting is to take a fine divided and sometimes dusty, unpalatable and difficult to handle feed material by using heat, moisture and pressure.

Generally, pellets will be formed in size from 10/64” o 48/64” or longer than the diameter but mostly the particles sizes smaller than 10/64” are desired because it more satisfactory from the standpoint of economics to produce a 10/64” or 12/64” pellet. Mostly livestock feeders agree pelleted fed better than a meal ration, this can be strengthen by several reasons which are the heat generated in conditioning and pelleting make the feedstuffs more digestible by breaking down the starches, the pellet simply puts the feed in a concentrated form and pelleting will minimizes waste during the eating process. Consequence, the animal will be more apt to receive a totally mixed ration than are that separated through these processes by feeding pelleted feed. In meanwhile, the bulk density will be increased which enhances storage capabilities of most bulk facilities.

In the production of animal feed, the process of producing food in the form of pellets (pelleting process) usually used to increase bulk density and improve their delivery of rations of feed. According to a research done by Kaliyan and Morey (2006), pellets usually have a diameter of 4.8-19 mm and a length of 12.7-25.4 mm and in cylindrical shape.

Pelleting process usually used pelletiser or a pellet mill for the whole process of pelletisation. Pellet mills with a large scale, consist of a die with annular framework of extrusions hole and a roller. Raw materials will be guided by the roller which serves as a repellent, and raw materials will be push through the die holes, and will be released as pellets forms. Then, the pellet will be cut with a knife according to the desired size. The resulting pellets are collected manually and packages according to customer requirements.

2.7 Important Parameter for Pelleting Process

In pellet production there have several important parameter that need to be taking care. They are types of raw materials, particle size, moisture content, conditioning temperature, pelletiser temperature, die length and diameter and die rotation speed (Stelte et al., 2010). The friction generated in the press channels of a pellet die during the process will produced a certain temperature and only some advanced, experimental set-ups allow a controlled heating or cooling. The friction in a pellet can be lowered by changing the die dimensions (i.e. length, diameter) (Nhuchhen et al., 2014) as well as moisture addition to the raw materials before pelletisation. Different studies have indicated that temperature increase results in better pellet quality and less friction during pelletisation (Rudolfsson et al., 2015).

The pelletisation process is a complex interaction between particles, their constituents and forces. Mani et al. (2002) reviewed the biomass pelleting process and the effect of various process parameters on pellet density and durability. Samson et al. (2005) reported the energy analysis and assessment of switch grass pelleting process. They found that switch grass pellet hardness moderately increased with a decrease in particle size from 3.2 to 2.8 mm. Water has been shown to act as a plasticiser, reducing the softening

temperature of lignin in pellet production and thus promoting the formation of an inter-particle network of molten and solidified lignin (Stelte et al., 2013). The water content is most important factor in pelletising process.

Next, length and diameter of the dies is one of the most crucial parameters for biomass pelletisation. The smaller diameters will show result in an increased surface to volume ration and provide a better transport of heat into and out of the pellet. This is because friction between the biomass particles within a pellet as well as between the press channel wall and the particles at the outside of the pellet. A smaller diameter will increase the surface and the number of particles touching the metal surface (Stelte, 2015).

From several studies, the die rotation speed indicate that it has been shown to be correlated to pellet quality because when the rotation speed of the die decreasing it will increase the pellet quality. The slower rotation results in a longer retention time of the pellets in the die while they are still under pressure and that may result in an improved softening and flow of polymers, forming an inter-particle network with more links and better stability as for pellets (Stelte, 2015).

2.8 Binder Process

Starch is a carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds usually contained in potatoes, corn, wheat, cassava, tapioca and rice, etc. Starch contains linear, helical amylose and branched amylopectin. Varying with plant species, starch generally contains 20% – 25% amylose and 75%-80% amylopectin. Starch can function as a thickening, stiffening or gluing agent when dissolved in warm water. Thus, adding a small amount of binder when making pellets is aimed to improve the pellet quality and avoiding decomposition and at the same time to reduce energy consumption, improve pellet making machine productivity and increase the service life of consumable spare parts like roller and die of a pellet mill.

The binder process is additional method that will support in pellet production for this research. In some research they have created the mixture for 1% vitamin tablets, sunflower oil 2%, egg albumen and tapioca flour 12% for binding and then 10% of boiled water was added and mixed well for 5 minute until the mixture made to a paste like form (Radhakrishnan et. al., 2013). The use of 30% tapioca in layer diets was also comparable to the conventional maize- soybean meal diet but when use of tapioca of 45% and 60% will be resulted in significantly lower layer performance.



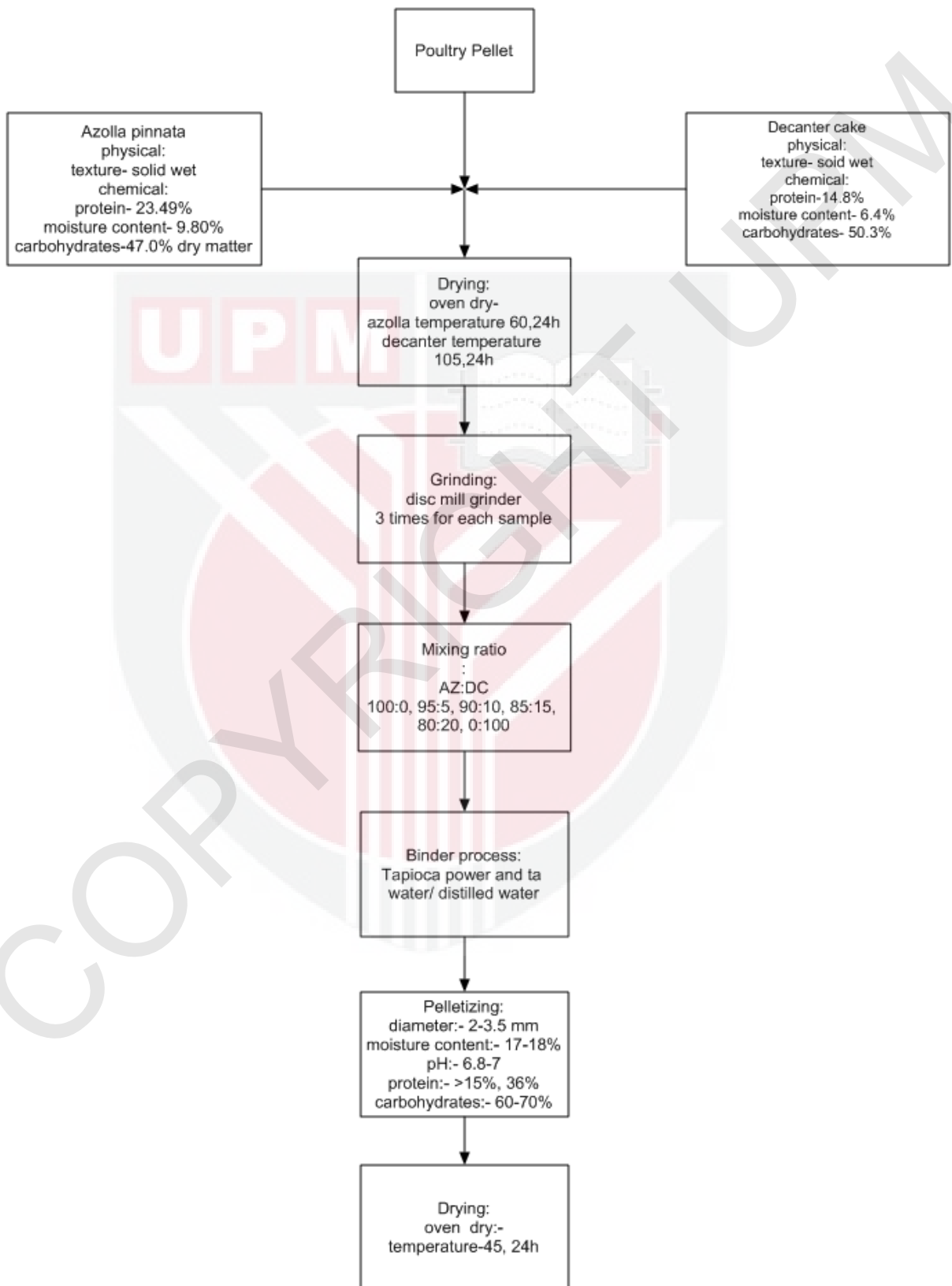
CHAPTER 3

METHODOLOGY

3.1 Introduction

The pellet feed was produced by combination of *Azolla pinnata* powder with decanter cake powder and the combination was mixed by selected ratio which is *Azolla pinnata* (AP) to Decanter cake (DC) ratio of 100:0, 95:5, 90:10, 85:15, 80:20 and 0:100. Firstly, the main ingredient for pellet feed which is AP was collected from Versmap rack in front of Food and Processing Engineering laboratory, UPM and the second ingredient which decanter cake was collected from palm oil mill at Jengka, Pahang. Then, both of ingredient were bind with tapioca powder and water to easy form the pellet in shape while give the moisture content for pellet. After that, the produced pellet has been fed to 18 chicks from 4/5/2019 until 1/6/2019 for 29 days of evaluation.

3.2 Flow Chart of Methodology



3.3 Materials

The preparation of pellet feed consists of two main raw ingredient which are *Azolla pinnata* and decanter cake; and the binder material used was tapioca powder and tap water.

3.3.1 Preparation of *Azolla pinnata*

In this study, *Azolla pinnata* has been used as main ingredient because of their higher content of protein (Figure 3.1). This material was prepared to be dried by sunlight at ambient around 36°C and until completely dried while for oven dry take around 24 hour at temperature 45°C or 60°C. The sunlight method take more time to dry material than oven dry because the sunlight drying process was depending on condition surrounding especially weather but using oven can faster the drying process and save more time production. However, the sunlight method more convenience to maintain the moisture content of material as in figure 3.2 and figure 3.3 show the different structure of dry *Azolla pinnata* between sunlight dry and oven dry.



Figure 3. 1: Raw *Azolla pinnata* sample before drying process



Figure 3. 2: *Azolla pinnata* sample by sunlight drying method



Figure 3. 3: *Azolla pinnata* sample by oven drying method

3.3.2 Preparation of Decanter cake

For this study decanter cake was coloured brown-blackish paste as figure 3.4 and also dried by sunlight dry method and oven dry method likes *Azolla pinnata* sample. This sample was dried under sunlight for 24 hour or 48 hour for completely dried at temperature around 36°C or decanter cake was dry using oven for 24 hour at temperature 60°C or 105°C.



Figure 3. 4: Decanter cake sample from palm oil mill Jengka, Pahang

3.3.3 Tapioca powder

Tapioca is a starch extracted from cassava root and it consists of almost pure carbs while contains very little protein, fiber or nutrients. So, tapioca powder has been use to bind both sample together to produce feed pellet.

3.3.4 Tap water/ Distilled water

The moisture content in pellet feed also important element as it will hold all macro-nutrients (Nitrogen, Phosphorus and Potassium) in ionized stage. In this study, clean water will be used during binder processing to form the pellet shape.

3.4 Pellet Feed Production

The pellet feed was produced by combining both sample which are AP to DC based on selected ratio of 100:0, 95:5, 90:10, 85:15 and 80:20. Firstly, the dried sample was grinded into powder form by using disc mill machine and this is because powder form make the mixing process become easier in pellet feed production. Then, both sample was sieved to separate thin powder from rough powder or dirt before being mixture together in selected ratio. Next, the powder sample being mixture into container based on selected ratio and added with 12% of tapioca powder and 10% of water. After that, the pellet feed

been form by mixing tapioca powder and waster in diameter around 2-3.5 mm as shown in (figure 3.7). The pellet feed been dry in oven for 24 hour at temperature 45°C to achieve require of moisture content for poultry feed consumption.



Figure 3. 5: *Azolla pinnata* after grinded by Disc Mill machine



Figure 3. 6: Decanter cake powder for pellet feed production



Figure 3. 7: Pellet feed produced from *Azolla pinnata* with decanter cake by selected ratio

3.5 Proximate Analysis

Proximate analysis is to estimate and determine the content of the major food components, which are moisture, lipids, proteins, ash, crude fibre that are contained in food. This study was using proximate analysis to analyse carbohydrate and protein content in pellet feed that has been produced. The pellet feed produced was analysed through DUMAS method that was conducted at ALS Technichem (M) Sdn Bhd Shah Alam, Malaysia. ALS is a global leader in the testing, inspection and certification industry that provides services to government, multi-national companies, manufacturers, raders, consultants and mining companies across the world.

As mentioned above, the analysis was conducted with DUMAS method which is a method of nitrogen determination by combustion that consists of the pyrolysis of the sample and determination of the amount of nitrogen through a cell of thermal conductivity (TDC). Samples of known quality are burned in a high temperature (about 900 ° C) chamber in the presence of oxygen. This results in the release of CO_2 , H_2O and N_2 , CO_2 and H_2O are removed by passing the gas through a special column that absorbs them. The nitrogen content is then measured by passing the remaining gas through a column that ultimately has a thermal conductivity detector. The column helps to separate nitrogen from any residual CO_2 and H_2O that remain in the gas stream. The instrument is calibrated by analyzing materials that are pure and have a known nitrogen concentration, such as EDTA (= 9.59% N). Therefore, the signal from the thermal conductivity detector can be converted to a nitrogen content. As with the Kjeldahl method, it is necessary to convert the nitrogen concentration in the sample to a protein content using a suitable conversion factor, which depends on the exact amino acid sequence of the protein. The result has been reported as shown in table 4.1 and the data was in g / 100g units.

3.6 Pellet Feeding

The experiment was performed in Bio-environmental Laboratory, Department of Biological and Agricultural Engineering. Eighteen chicks of 4 week old were used in this experiment (Fig. 3.8) . The birds were identified and then individually weighed initially, and then divided into 6 groups. They were allocated to 6 treatments per treatment, and 3 birds per group (Fig. 3.9). The initial total body weight of the chicks in each treatment was weighted. The experimental period was preceded by a preliminary period of one week. The chicks were fed a commercial type starter diet, and then were allocated at random to one of the experimental diets.

The six dietary was classified as maize (common feed for chick) and followed by selected ratio of 100:0, 95:5, 90:10, 85:15 and 80:20 as T1 until T6. The requirement for chick in container was prepared such as by 25W bulb for lighting, adequate ventilation was used to ensured and to make the chick comfortable, the feeding and water troughs were cleaned daily to ensure there was no contamination and the floor was covered with wood shavings to act as absorbent for the faecal droppings.



Figure 3. 8: Chick used for experimental analysis



Figure 3. 9: Container used as chick shelter

3.6.1 Data Collection

The weight of chicks were measured before fed with pellet as original weight. Then, each chick was weighed twice daily; 9.00 am in the morning and 5 pm. The weight increment for 29 days was calculated and compared along the experiment running.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Proximate Analysis

High moisture content may induce microbial growth which is undesirable in feed pelleting. Thus, the decanter cake and *Azolla pinnata* has been kept dry for long-term storage and further processing. In this study, decanter cake and *A.pinnata* samples were dried and estimated the moisture content simultaneously before storage in an air-tight container at room temperature for compositional analysis. Table 1 shows the composition of pelletized dried decanter cake and *A.pinnata*.

It was observed that the carbohydrate content is more than 84% as compared to the protein content at the range 14-31%). The addition of decanter cake has tremendously increased the protein content while lower the carbohydrate content.

<i>Azolla pinnata</i> to decanter cake ratio	Carbohydrate content, g/100g (%)	Protein content, g/100g (%)
100:0	39.9 (86)	6.3 (14)
95:5	40.2 (85)	7.1 (15)
90:10	30.4 (84.9)	5.4 (15.1)
85:15	43.6 (84.3)	8.1 (15.7)
80:20	37.6 (84.1)	7.1 (15.9)
0:100	21.6 (68.7)	9.9 (31.3)

Table 4. 1: Carbohydrates and proteins content in selected pellet ratio

Azolla pinnata differences in nutrient composition may be due chemical composition of water nutrients and also may be due to differences in environmental conditions such as respond to heat, light intensity and its resulting impact on their growth and morphology.

The mixing ratio of AP and DC with the tapioca binder could has an effect in the carbohydrate and protein content.

4.2 Body Weight Gain

The result were analysed based on the weight of chicks for 29 days. The percentage of increment weight of chick were shown in table 4.2 and that data was calculated as shown in appendix 3. The chicks in this study fed with different inclusion levels of dried *A. pinnata* and decanter cake indicate the acceptable replacement level. Less than 20% of decanter cake in the diet significantly increased the growth of chicks. It was observed that the weight increment percentage was the lowest in chicks fed with maize only (control) of 21% weight increment. There was slightly difference in weight increment percentage between the fish fed treatments T1, T2 and T4. On the other hand, the chicks treatment in T3 and T5 were significantly higher than the other treatments with 95.5% and 193.4% of weight gained. However, 5% increment of the decanter cake in the diet has no conclusive pattern. For example, at no decanter cake the weight (T1) increased up to 46% and with 5% decanter cake (T2) has 39% weight increment while 20% decanter cake (T5) could tremendously increase the chicks weight up to 193%. The other study by Mousumi (2018) reported that replacement fish meal with *Azolla pinnata* at 0%, 50%, 75% and 100% to replace soybean meal has effect a significant reduction of fish growth. The poorest performance was observed in fish fed completely with *A. pinnata*. They concluded that inclusion of 25% of *A.pinnata* could be used in fish diet without significantly lowering the growth and product quality. In this study, we formulated the *A.pinnata* more than 80% with 5% differences at each treatment. It could be observed that more inclusion of *A. pinnata* has decreased the percentage of chicks weight with 193% for 80% *Azolla pinnata* as compared to 95% *Azolla pinnata* with 39.3% weight gained. This could be due to pellet feed produced from AP with DC which has balance nutrient requirement for poultry feed which was not measured in this work. The proximate analysis of carbohydrate and protein has no significant difference between the

AP and DC ratio used. However, as decanter cake inclusion increased in diet, the higher protein content was observed (Table 4.1) .

Pellet at AP: DC Ratio	Increment weight of chick (%)
Maize (control)	21.02
100:0 (T1)	46.54
95:5 (T2)	39.31
90:10 (T3)	95.50
85:15 (T4)	30.96
80:20 (T5)	193.44

Table 4. 2: Increment weight of chick during pellet feed evaluation

The average weight of chicks for 29 days were as presented in Table 4.3. The coefficient of variation (CV) is a statistical measure of the dispersion of data points in a data series around the mean and them is represents the ratio of the standard deviation to the mean, and it is a useful for comparing the degree of variation from one data series. The lower value of standard deviation and CV was observed.

Pellet at AP:DC ratio	Mean/Average (g)	Variance	Standard Deviation	CV
Maize	141.20	51.75	7.19	5.10
100:0	132.02	105.35	10.26	7.77
95:5	140.75	133.32	11.55	8.20
90:10	113.92	261.37	16.17	14.19
85:15	98.59	19.55	4.42	4.49
80:20	153.83	81.37	9.02	5.86

Table 4. 3: Statically analysis for six dietary

The body weight of chicks for daily measurement was plotted as in figure 4.1. The chicks were fed with pellet feed ratio from day first for a week with alternate maize. The chick was continued been fed with pellet feed ratio from day eight until day 29th. In general, all the treatment has resulted in the weight increment.

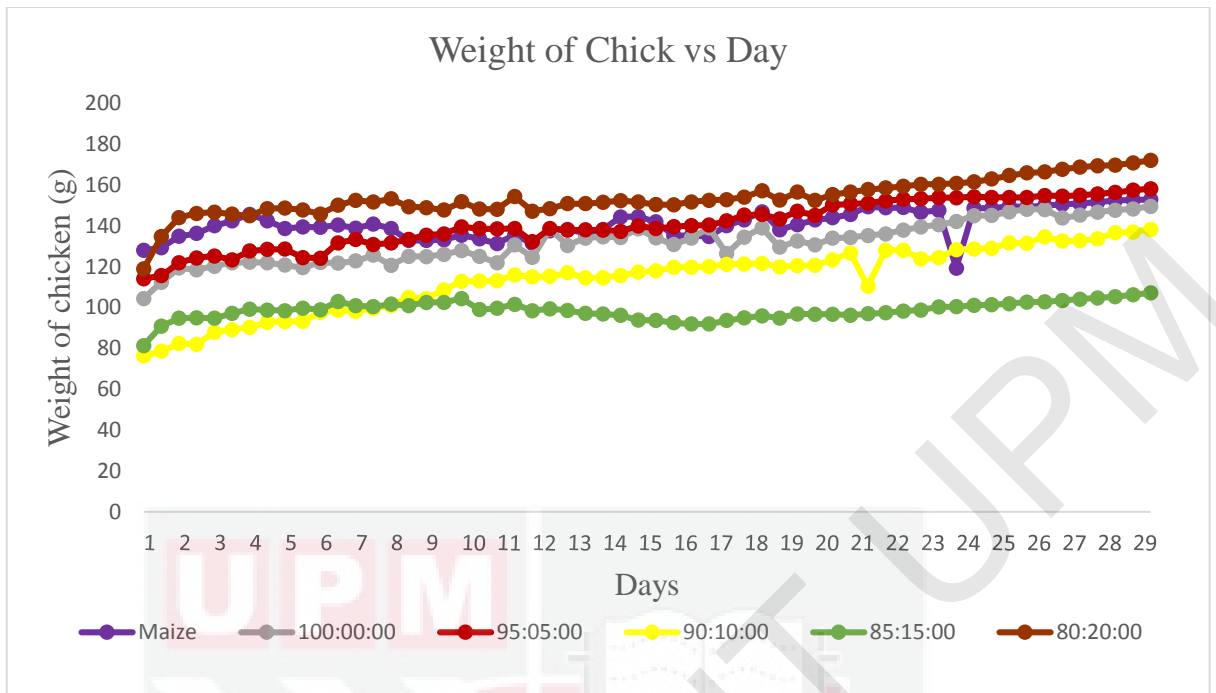


Figure 4. 1: Weight of chick based on evaluation day for 6 dietary

Interestingly, as observed in figure 4.2 the diet intake of experimental chicks supplemented maize was almost similar compared to that for chicks fed with *A.pinnata* pellet only (100:0) although both have good increment weight of chicks. It shows that 100% *Azolla pinnata* could replace maize as food supplement.

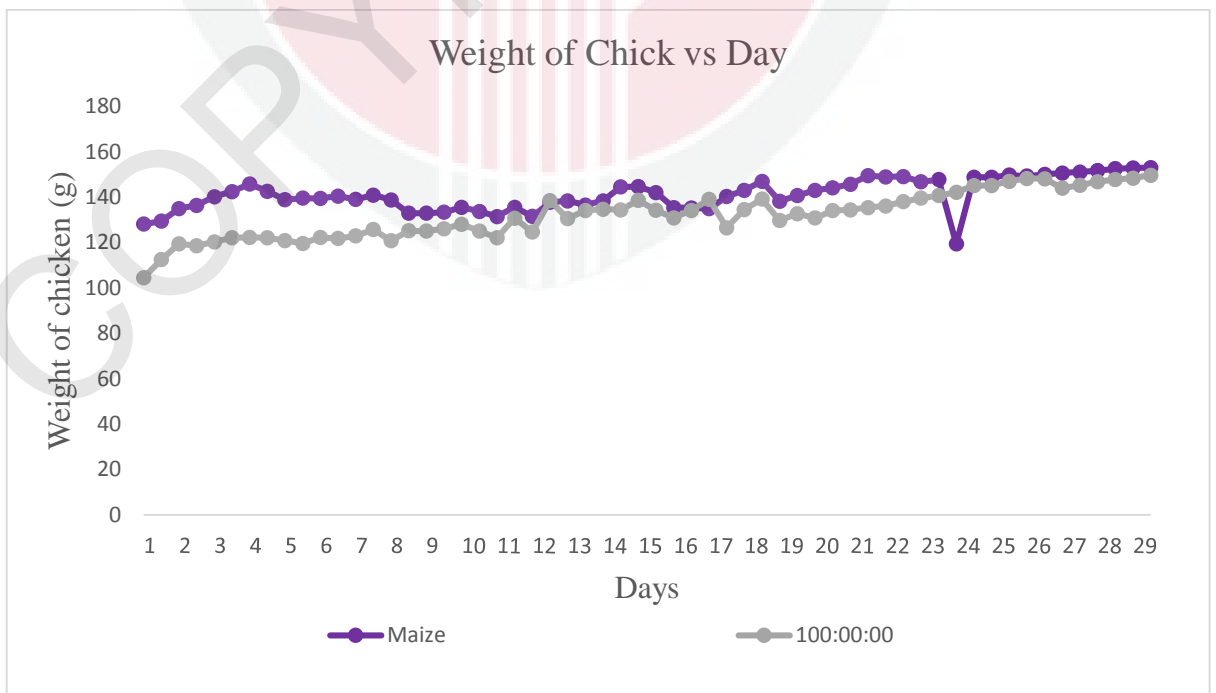


Figure 4. 2: Different weight of chick between maize and 100:0

Similar observation was noted as in figure 4.3 which show the comparison weight of chick between maize and pellet feed ratio of 95:5. The diet intakes with the level of DC increased to 5%, has no significant difference weight in intake between supplemented chicks with maize.

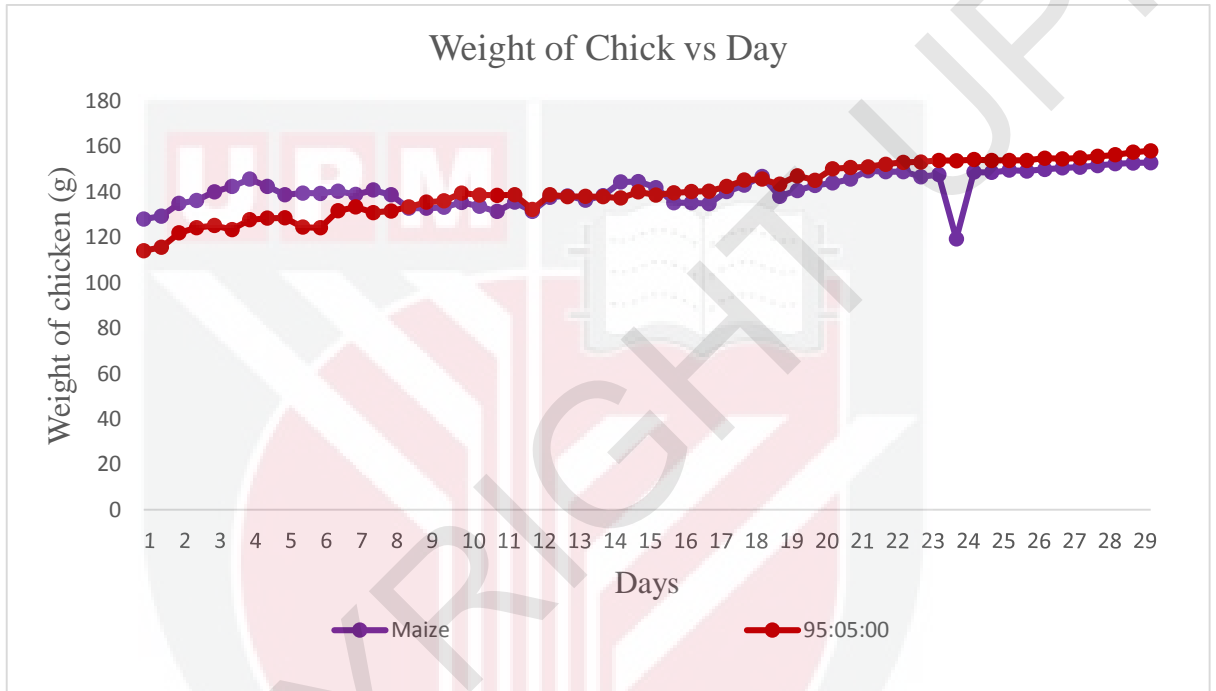


Figure 4. 3: Different weight of chick between maize and 95:5

Figure 4.4 shows the comparison between maize only and pellet feed ratio (90:10). The pellet feed ratio with 90% of AP to 10% of DC has increased the weight of chicks at about 95%. The weight has consistently increased in chicks as compared to maize fed that have almost constant weight per day.

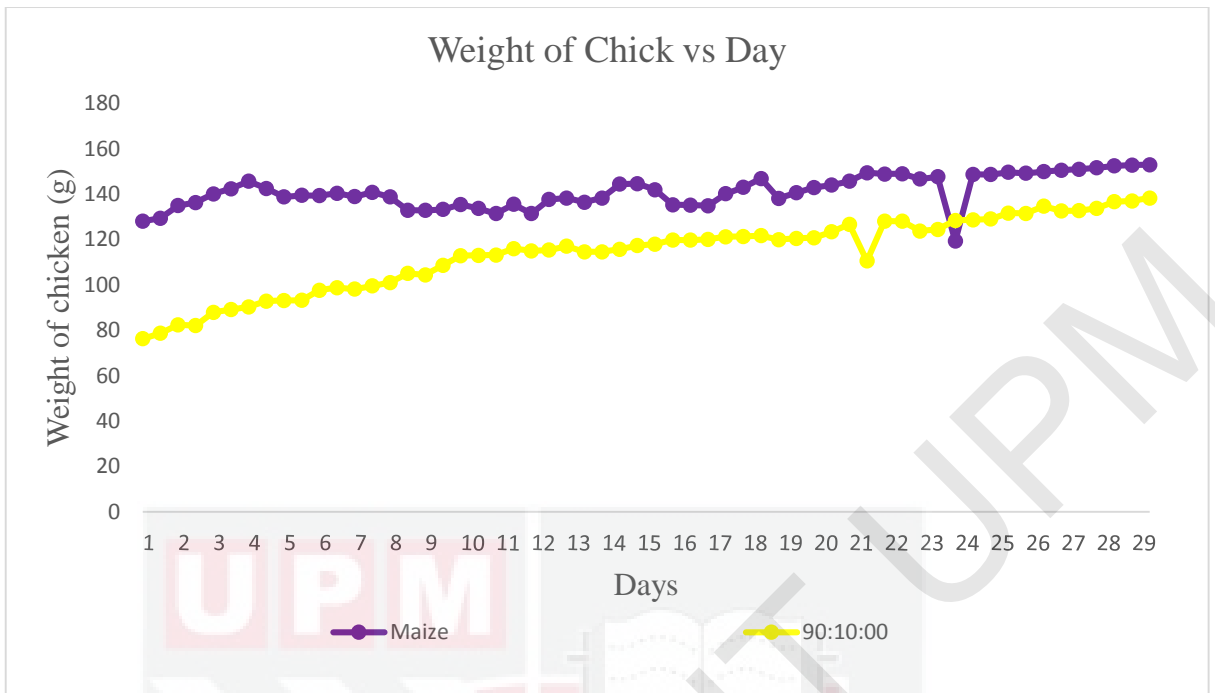


Figure 4. 4: Different weight of chick between maize and 90:10

Similar pattern was observed in figure 4.5 that show the chicks weight for pellet feed ratio at 10% DC and 90% AP dietary also has consistent weight increment along the experiment than maize dietary that has more reduction in weight.

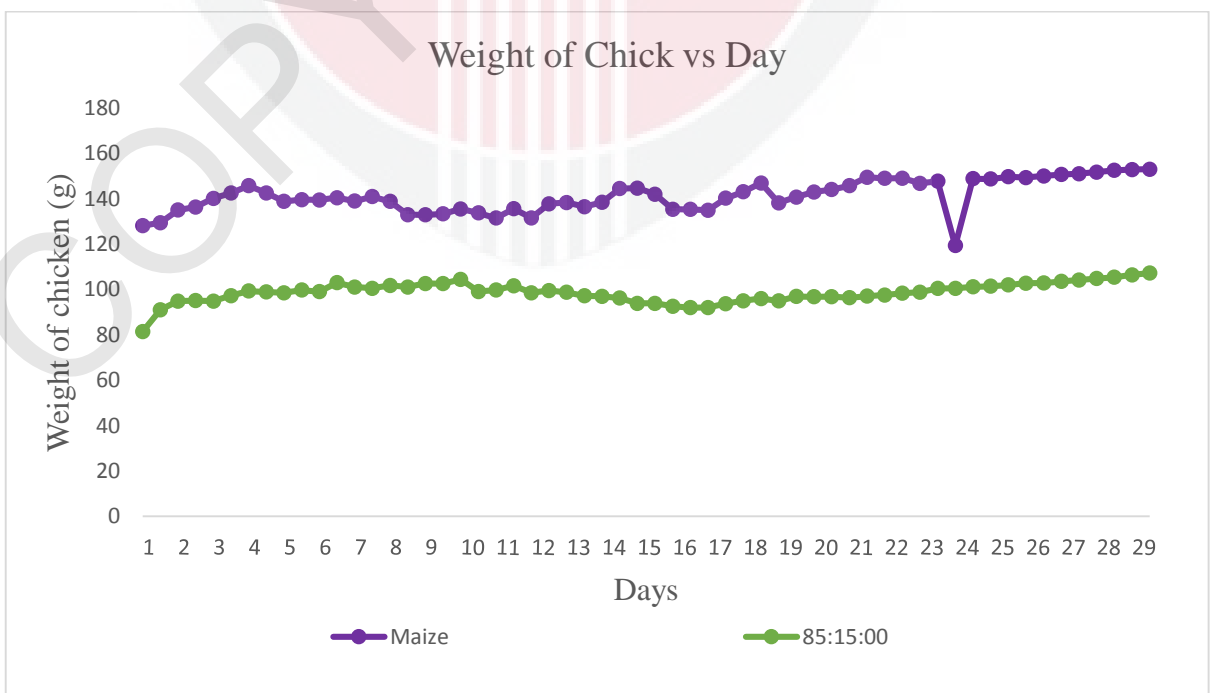


Figure 4. 5: Different weight of chick between maize and 85:15

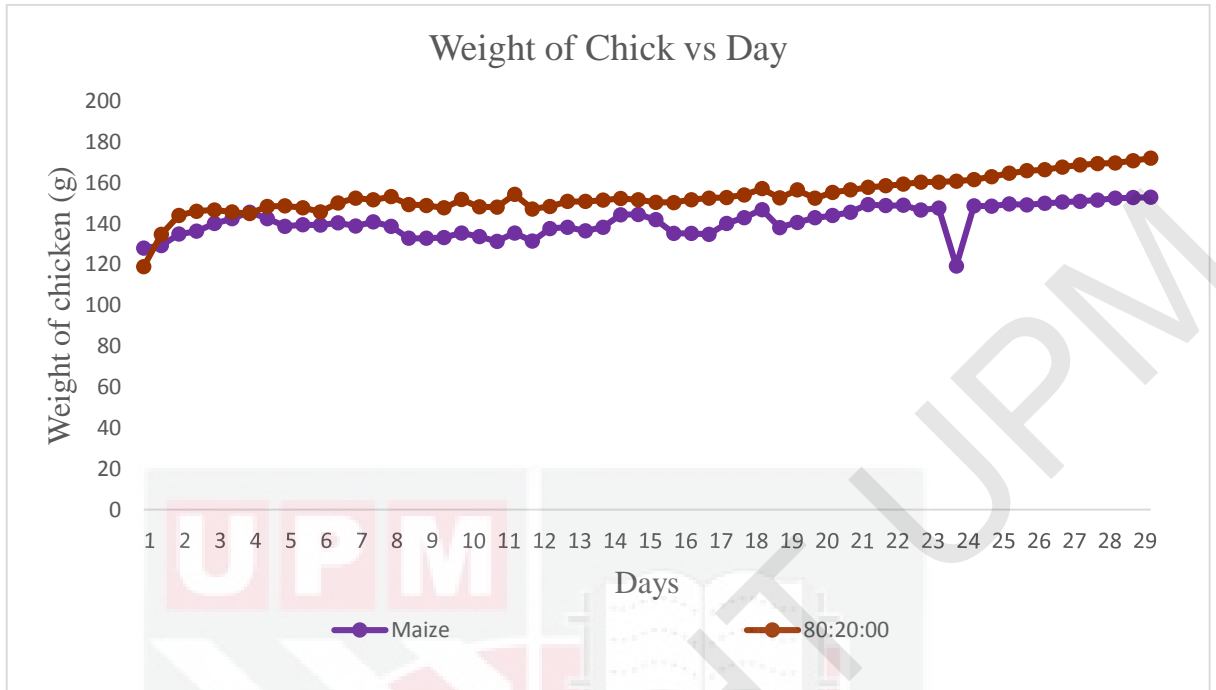


Figure 4. 6: Different weight of chick between maize and 80:20

Based on all graph, dietary feed plot for ratio of 95:5 and 80:20 has higher weight increment than other treatment mainly as compared to maize dietary. This is because of protein and carbohydrate content in both dietary has achieve the consumption requirement for poultry feed. The inclusion of decanter cake up to 20% has improved the weight of chicks daily. Although on proximate analysis all dietary has almost same percentages of protein which is 15% and carbohydrate percentage 84%.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Azolla pinnata and decanter cake have a potential as a promising and economical feed for poultry feed. This study show that combination of *Azolla pinnata* with agricultural byproducts such as decanter cake can improve weight of poultry and thus production performance of animals. Thus, *Azolla pinnata* has future as a potential feed ingredient for poultry feed. It can be concluded that inclusion of decanter cake up to 20% of poultry diets with 80% of *Azolla pinnata* is the best formulation for the highest weight increment.

The main ingredient of *Azolla pinnata* and decanter used to produce pellet feed for poultry consumption can be an economical option for poultry industry and also farmers. This is because *Azolla pinnata* is a plant that easy to breed and grow while it can be widely distributed in any watery space /area. Besides that, decanter cake also easily be obtained as palm oil mill waste.

5.2 Recommendations

At the end of this research, few parameters were not taken into consideration. Therefore, in order to improve the outcome in the future, few recommendations are listed as below:

- a) To include cost analysis for the optimize formulation obtained.
- b) To include different ratio of *Azolla pinnata* to decanter cake such around 50% below so can see more impact and effect to animal growth while compared with normal feed (maize). In this study the ratio used was 80% and above for *Azolla*.
- c) To include the proximate analysis at each different ratio of pellet used.
- d) To include more analysis such as ;
 - ❖ $weight\ gain\ (g) = Mean\ final\ weight\ (g) - Mean\ initial\ weight\ (g)$,
 - ❖ $specific\ growth\ rate\ (SGR)(\% \ day^{-1}) =$
 $(L_n\ final\ weight\ (g) - L_n\ initial\ weight\ (g)) / (Period\ in\ days) \times 100$,
 - ❖ $Feed\ conversion\ ratio\ (FCR) = Feed\ intake\ (g) / Live\ weight\ gain\ (g)$,
 - ❖ $Condition\ Factor\ (K) = Fish\ weight\ (g) / (Fish\ length)^3\ (cm) \times 100$
 - ❖ $Hepatosomatic\ index\ (HSI) = Liver\ weight\ (g) / Body\ weight\ (g) \times 100$
 - ❖ $Protein\ efficiency\ ratio\ (PER) = Weight\ gain\ (g) / Protein\ intake\ (g)$
 - ❖ $Net\ Profit = Total\ income - Total\ cost$

Furthermore, through the net profit can see the comparison of production cost for poultry between normal feed (maize) and pellet feed produced. Therefore, the production cost for poultry industry will be improved with pellet feed produced while reducing the production cost or give negative impact to poultry industry.

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APPENDIX

Appendix 1: Equipment for sample of preparation.



Oven for drying process- UN55.



Grinder machine- Disc Mill

Appendix 2: Daily weight of chick

DAY	MAIZE	100:00:00	95:05:00	90:10:00	85:15:00	80:20:00
1	128.063	104.45	114.14	76.32	81.38	118.92
	129.367	112.42	115.65	78.67	91.01	134.86
2	134.933	119.43	122.00	82.42	94.85	144.12
	136.3	118.52	124.34	82.06	95.08	146.10
3	140.12	120.24	125.25	87.94	94.83	146.74
	142.4	122.00	123.43	89.17	97.20	145.71
4	145.677	122.20	127.74	90.32	99.26	144.98
	142.5	122.00	128.47	92.88	98.83	148.50
5	138.79	120.80	128.64	93.16	98.53	148.68
	139.5	119.55	124.50	93.23	99.73	147.85
6	139.363	122.13	124.20	97.70	98.97	145.78
	140.4	121.73	131.69	98.81	102.93	150.10
7	138.9	122.87	133.37	98.15	101.03	152.53
	140.843	125.65	130.91	99.58	100.48	151.67
8	138.743	120.72	131.62	101.03	101.71	153.27
	132.903	125.10	133.40	105.06	101.04	149.38
9	132.867	125.00	135.48	104.43	102.59	148.95
	133.283	126.03	136.07	108.65	102.54	147.73
10	135.467	127.93	139.51	112.92	104.42	151.83
	133.667	125.02	138.70	113.00	99.07	148.23
11	131.417	122.03	138.52	113.21	99.70	148.13
	135.5	130.70	138.74	115.99	101.63	154.31
12	131.467	124.57	132.33	115.03	98.53	147.25
	137.667	138.43	138.77	115.33	99.47	148.45
13	138.287	130.45	137.97	117.07	98.68	150.88
	136.45	134.08	138.02	114.58	97.20	150.87
14	138.31	134.67	137.94	114.60	96.95	151.54
	144.4	134.33	137.37	115.75	96.20	152.38
15	144.587	138.50	140.00	117.36	93.84	151.70
	141.933	134.17	138.67	117.97	93.83	150.47
16	135.333	130.82	139.63	119.80	92.60	150.38
	135.2	134.07	140.13	119.73	91.96	151.77
17	134.867	138.97	140.40	120.10	92.02	152.53
	140.167	126.43	142.45	121.23	93.70	152.87
18	142.967	134.50	145.25	121.37	94.97	154.10
	146.867	139.07	145.64	121.70	95.96	157.19
19	138.15	129.60	143.39	119.95	94.89	152.63
	140.637	132.63	147.18	120.51	96.90	156.50
20	142.933	130.77	145.20	120.78	96.73	152.48

	144.007	134.00	150.27	123.37	96.80	155.37
21	145.647	134.33	150.77	126.70	96.33	156.57
	149.4	135.33	151.10	110.57	97.00	157.80
22	148.883	136.07	152.23	128.13	97.50	158.57
	149	137.97	153.07	128.07	98.30	159.33
23	146.7	139.47	153.23	123.77	98.80	160.37
	147.667	140.60	153.90	124.43	100.40	160.40
24	119.333	142.13	153.80	128.40	100.47	160.83
	148.733	145.03	154.27	128.70	101.13	161.57
25	148.633	145.10	153.93	129.03	101.40	163.00
	149.633	146.93	153.93	131.63	101.93	164.70
26	149.233	148.17	153.90	131.53	102.73	165.93
	149.933	148.00	154.87	134.67	102.83	166.40
27	150.567	143.87	154.60	132.67	103.53	167.77
	150.967	145.23	155.00	132.70	104.17	168.75
28	151.633	146.77	155.67	133.77	104.77	169.50
	152.433	147.70	156.40	136.66	105.33	169.73
29	152.767	148.33	157.50	137.00	106.37	170.90
	152.933	149.60	158.17	138.27	107.17	172.07

Appendix 3: Chick weight increment calculation

Chick Weight Increment (%)

$$= \frac{\text{Final Weight of Chick (g)} - \text{First Weight of Chick (g)}}{\text{Original Weight of Chick (g)}} \times 100$$

Statically Analysis:

Mean:

$$\bar{X} = \frac{\sum X}{N}$$

Variance:

$$s^2 = \frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}$$

Standard Deviation:

$$\sqrt{\frac{\sum |x - \bar{x}|^2}{n}}$$

CV:

$$CV (\%) = \left(\frac{\text{Standard deviation}}{\text{Mean}} \right) \times 100$$

Appendix 4: Carbohydrate and protein analysis result from ALS laboratory

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Analytical Results

MISCELLANEOUS
 001 AZOLLA:DECANTER CAKE 100:0

Test description	Method	LOR	Unit	Result
Nutritional Panel				
Carbohydrates - Total	OF035	0.1	g/100 g	39.9
Protein	OF119.AOAC972.43	0.1	g/100 g	6.3

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MISCELLANEOUS
 002 AZOLLA:DECANTER 95:5

Test description	Method	LOR	Unit	Result
Nutritional Panel				
Carbohydrates - Total	OF035	0.1	g/100 g	40.2
Protein	OF119.AOAC972.43	0.1	g/100 g	7.1

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MISCELLANEOUS
 003 AZOLLA:DECANTER 90:10

Test description	Method	LOR	Unit	Result
Nutritional Panel				
Carbohydrates - Total	OF035	0.1	g/100 g	30.4
Protein	OF119.AOAC972.43	0.1	g/100 g	5.4

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MISCELLANEOUS
 004 AZOLLA:DECANTER 85:15

Test description	Method	LOR	Unit	Result
Nutritional Panel				
Carbohydrates - Total	OF035	0.1	g/100 g	43.6
Protein	OF119.AOAC972.43	0.1	g/100 g	8.1

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MISCELLANEOUS
005 AZOLLA:DECANTER 80:20

<i>Test description</i>	<i>Method</i>	<i>LOR</i>	<i>Unit</i>	<i>Result</i>
Nutritional Panel				
Carbohydrates - Total	OF035	0.1	g/100 g	37.6
Protein	OF119.AOAC972.43	0.1	g/100 g	7.1

