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Master's Thesis 2021 30 ECTS
Faculty of Biosciences
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Characterization of functional physical properties of the dry petfood manufactured with animal, plant, and microalgae ingredients.

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Abstract

The aim of the thesis is to gain better understanding on the effects of defatted microalgae biomass (*Desmodesmus sp.)*, vital wheat gluten and potato protein on physical qualities of extruded dry pet food. Two experiments were conducted to investigate the effects. In the first experiment, microalgae (DMG) replaced poultry meal partially, at inclusion rate of 10%, 15% and 20%. Higher inclusion rates of DMG resulted in lower moisture content, lower water activity, but higher hardness and durability. Increasing extrusion water rate and decreasing screw speed improves expansion of pellets made with 15% and 20% DMG. Only extruded pellets produced with 20% DMG achieved acceptable water activity (< 0.6). In the second experiment, poultry meal is replaced with vital wheat gluten and potato protein at ratio 1:1. The experiment showed that the plant proteins improved expansion ratio, hardness, and durability but extruded pellets have high moisture content and unacceptably high-water activity (both were > 0.6).

The results obtained from the thesis shows that microalgae biomass, vital wheat gluten and potato protein may be used in place of animal protein to improve physical qualities of extruded feeds, especially pet food.

Keywords: extrusion, *Desmodesmus* sp., microalgae, vital wheat gluten, potato protein, physical quality, expansion ratio, moisture content, water activity, hardness, pellet durability index, pet food.

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Abbreviations

DMG	Defatted biomass of *Desmodesmus* sp.
WP	Vital wheat protein
PT	Potato protein
PDI	Pellet durability index
SME	Specific mechanical energy

1. Introduction

1.1. Pet food industry and its growing market

Pet food is a fast-growing industry, even during the COVID-19 pandemic.

According to BusinessWire (2021), the global pet food market would continue to grow in 2021 at a compound annual growth rate (CAGR) of 4.5%. North America was the largest region in the market of 2020, Western Europe was the second largest, and Middle East was the smallest.

According to Euromonitor International, 2020 witnessed the growth of wet food, dry food, and pet treat segments in the market. Among these, pet treat had the highest growth (>10%), dry food came second (>8%) and wet food is at the third place (about 8%). There would be premiumization trends in pet food innovation and production. Less processed pet food products (fresh, semi-moist…) are also favored among consumers. Functional products gain more attention from consumers (EuromonitorInternational, 2018). The former two trends were observed at the Global Pet Expo 2020 (Tyler, 2020)

It should also be noted that the mentioned trends have little to do with the consumers' decision to purchase pet food. In late 2020, a study in Korea identified major factors that affect their behaviors are 1) brand reputation and 2) healthiness perception (Kwak & Cha, 2021). The same study emphasized that consumers are willing to pay for a pet food product and recommend it to other people based on the brand reputation and their healthiness perceptions. Therefore, it can be inferred that the marketing game play a significant role in the pet food market. However, more studies on customers' behavior should be conducted in other regions to gain a better picture on this matter.

1.2. Pet nutrition (focus on dog and cats)

1.2.1. Canine and feline nutrition

Dogs are omnivores, and cats are carnivores. When dogs and cats were domesticated by humans, they evolved to adapt with different diets, hence the difference in their nutrition requirements.

The domestication of dogs happened even before the domestication of livestock. They were the first animal species that humans selectively bred and domesticated from wolves. They were our guards, hunting partners, shepherds and pets. (Peters *et al.*, 2005; Vilà *et al.*, 1997). Dog domestication results in their adaption for a starch-rich diet (Axelsson *et* *al.*, 2013), which might be what their early owners can provide (Clutton-Brock, 1990; Hemmer, 1990; Zeuner, 1963).

On the other hand, cats came to live with humans much later compared to dogs. As a result of the successful Neolithic agriculture revolution, houses and farms and food storages were built. The settlement attracts unwanted animals, especially rodents, to trash dumps and grain storages. These rodents provided a stable food source to wildcats (Driscoll *et al.*, 2009; Serpell, 2000; Sunquist & Sunquist, 2017). Cats were eventually fully domesticated only about 4000 years ago (Serpell, 2000). Since then, domestic cats have been adapting to a less strictly carnivorous diet (Darwin, 2010), but they are still obligate carnivores at the present.

Dogs and cats share similar essential amino acids and essential fatty acids requirements. The essential and non-essential amino acids in their diet are listed in Table 1. For fatty acids, they require omega-6 and omega-3 fatty acids (linoleic acid and alpha-linolenic acid e.g., respectively) in their diet; however, cats also need dietary arachidonic acid (Case *et al.*, 2010). Another interesting similarity is that both dogs and cats share their low tolerance for lactose and sucrose (Case *et al.*, 2010). Their lactase activity decreases as they age, therefore mature dogs and cats cannot tolerate lactose well. On the contrary, their sucrose activity is low when they are young, so sucrose is not recommended in formulations for puppies and kittens .

Table 1: Essential and non-essential amino acids for dogs and cats (Case et al., 2010)

Essential	**Non-essential**
○ Arginine ○ Histidine ○ Isoleucine ○ Leucine ○ Lysine ○ Methionine ○ Phenylalanine ○ Taurine (cats only) ○ Threonine ○ Tryptophan ○ Valine	○ Alanine ○ Asparagine ○ Aspartate ○ Cysteine ○ Glutamate ○ Glutamine ○ Glycine ○ Hydroxylysine ○ Hydroxyproline ○ Proline ○ Serine ○ Tyrosine

To explain the differences in their diet, we need to keep in mind that dogs are omnivorous while cats are carnivorous. For this reason, their ability to metabolize different nutrients (especially carbohydrate) are also different, and same thing can be said about their nutritional needs.

As omnivores, they do not mind a high-carbohydrate diet, if enough protein, fat, and other essential nutrients are included. They have all required enzymes to digest carbohydrate and to convert nutrient precursors to meet their nutritional demand. They also have sufficient activity of glucokinase to regulate the surge in blood glucose when consuming a high load of carbohydrate. Despite that, digestible carbohydrate is not essential in their diet, although pregnant females would require dietary carbohydrate for optimal reproductive performance (Romsos *et al.*, 1981).

Just like dogs, cats can digest fully cooked starch, yet it does not mean they can live healthily on a diet as omnivorous as dogs'. First, a high dietary carbohydrate intake would do more harm than good to them(Kienzle, 1994a; Kienzle, 1994b; Zoran, 2002). Secondly, cats do not have all essential enzymes to convert nutrients in plant-based material to their need. For this reason, cats need to consume animal flesh to get these nutrients directly from their food:

- Vitamin A (they cannot convert from beta-carotene).
- Arachidonic acid (they cannot convert from linoleic acids).
- Niacin (they cannot convert from tryptophan).
- Taurine is an essential amino acid for cats.

To put it simply, cats benefit from a high-protein, animal-based diet. Theoretically, it is possible to develop a vegetarian/ vegan cat food; but the formulation for such unconventional diets must be closely monitored to ensure they meet the nutrients need of the animal.

1.2.2. Types of (cooked) pet food

There are three commercial types of cooked pet food: moist food (wet food), dry food, semi-moist and soft-dry food. These types are differentiated by the moisture content, and (Crane *et al.*, 2010).

Moist food, also called wet food/canned food (because it is usually canned, but some are also packaged in pouches). The moisture content of moist food can be anywhere from 60% to more than 87% (Crane *et al.*, 2010). Typical moist food contains thickeners, such as gelatin to keep the food in its container's shape. Some moist foods are marketed as "loaf in sauce" accordingly how they look. Others are promoted as "meat in gravy", which appears to contain “meaty” chunks in gelled broth. In many cases, these chunks do have meaty texture, but they are not really meat but only extrudate from vegetable protein, starch, meat meal,… Many moist food brands provide feeding guide for moist food as the sole source for food and as a supplementary to dry food, and let the consumers decide how to feed their pets. In general, moist pet foods are highly palatable, and are usually promoted as a healthy way to feed the animals while keeping them hydrated. They are also more expensive than other types of pet food because of high moisture content and low dry matter.

In contrast to moist foods, dry foods have much lower moisture content (3% - 11%) and higher dry matter (Crane *et al*., 2010). Dry food is cheaper than moist food, however they are less preferred by pets. Most of the time, dry food brands’ feeding guide indicate that dry pet food can be used as the only food source but should be supplemented with moist foods. WALTHAM™ Centre for Pet Nutrition (pet science centre for Mars Petcare) also recommends feeding a mixed diet where 50% of calories come from wet food and 50% from dry food.

Semi-moist or soft-dry food are the hybrids of moist food and dry food. The difference between “semi-moist” and “semi-dry” is not clear, sometimes these terms are used interchangeably with one another. The moisture content of these pet foods is about 25% to 35%. They are usually packed in pouches like dry food and are expected to last for a longer time than wet food. To prolong shelf life, humectants and organic acids are added to reduce water activity and prevent mold (Crane *et al*., 2010). They are highly palatable, and can used as a food source, or pet treats, or topping to dry food to increase palatability.

1.2.3. Unconventional diets: vegetarian and hypoallergic pet food

Aside from health reason, one of the most common causes for vegans and vegetarians to quit meat and other animal products is to prevent cruelty to animals, and they associate meat with disgust, animal killing and cruelty (Rozin *et al*., 1997). The ethics–driven vegans and vegetarians clearly have great concern for animal welfare, and some even express their compassion toward animal by pet ownership. In most cases, they believe that pets (dogs and cats) are not meant to have vegetarian diets but feeding their pets an animal-diet goes against their dietary commitment (Rothgerber, 2013). In fact, many commercial vegan pet food products do not provide the animals enough nutrients to meet their needs (Zafalon *et al*., 2020).

In another research by Rothgerber (2014), vegans tried to resolve their dilemma by feeding dogs less animal-products dogs and trying to justify that a vegetarian diet is inappropriate for cats. Either ways, it is hardly a success: their pets still consume animal products, their guilt is lessened but persists, and the dilemma is not completely settled (Rothgerber, 2014). Understandably, ethical vegans and vegetarians are interested in exploring possible alternative, unconventional options (Rothgerber, 2013), which suggests potential for vegetarian diets in the pet food market.

Dogs and cats suffer from food allergies just like people, but there is a lack of literature on this matter. So far, the best treatment for their food allergies is avoiding the food allergens completely – but it is difficult to diagnose the food allergens because reliable diagnostic tests for allergies (on dogs and cats) are not widely available (Verlinden *et al*., 2006). Common food allergens in dogs are animal products form beef, chicken, milk, eggs, corn, wheat, and soy (White, 1988); and food allergy in cats are usually linked to dairy and fish proteins (Bhagat *et al*., 2017). These allergens are common animal-derived protein source in pet food, for that reason the novel protein ingredients would give pet food producers more flexibility for hypoallergic pet food formulations.

1.3. Novel ingredients in pet food industry

1.3.1. Pet food sustainability and its environmental impacts

1.3.1.1. Conventional ingredients in pet food

To simplify this topic, we will discuss only three major groups of ingredients in pet food: carbohydrate and fiber ingredients, protein ingredients, fats, and oils.

Carbohydrate is a cheap and important energy source in pet food. The most common source of carbohydrate for pet food are grains, such as corn, wheat, rice, barley,… For grain-free diets, pet food producers opt for tubers, roots, and legumes (peas, chickpeas,…) instead. Whole grains and legumes typically also provide a good amount of dietary fiber to pet food diets. However, additional fiber sources are usually added to promote intestinal health and reduce cost further because they are mostly by-products from food industry, like wheat bran, pea fiber, soybean hulls, rice hulls, beet pulp,…

Protein is not only the building blocks to the animals' body, but also an energy source for true carnivores like cats. Protein ingredients in pet food can be animal-based, plant-based or microorganism-based. Animal-based ingredients commonly used in pet food are mostly by-products from slaughterhouse and fish meal. Premium products often include meat instead of meat meal, such as beef, lamb, bison, venison,… The most common plant-based proteins are corn gluten meal and soybean protein (in forms of soybean meal and soy flour). Brewer's yeast and grain distillers dried yeast are the most microorganism-based protein in pet food, other microbe proteins like bacterial meal and microalgae meal are still being researched.

Animal-derived products have much higher carbon footprint than plant-derived products (Murphy-Bokern, 2010) or microorganism (Matassa *et al*., 2016; Olguín, 2012). While most of animal-based protein ingredients used in pet food are by-products of the food industry, the inclusion of meat in premium pet food has the biggest influence on the environmental impacts of pet food industry (Alexander *et al*., 2020). While the premiumization trends in pet food production does not help, there is a need for novel protein ingredients from plants and microorganism to combat the negative environmental impacts of the industry.

Fats and oils in pet food are mainly from terrestrial animal fat, fish oil and the fat content of animal-derived products such as poultry-meal and fishmeal. Animal fats are normally preserved with either tocopherols or a combination of BHA and citric acid to prevent oxidation. Fish oil is produced unsustainably while its price is increasing (De Silva *et al*., 2011). Vegetable oil is also used in pet food to supplement omega-3 fatty acids.

1.3.1.2. Pet food sustainability

The environmental impacts of pet food industry somehow has been overlooked with very little address from both scientists and fisheries (De Silva & Turchini, 2008). Pet food industry uses a lot of by-products from food industry (Swanson *et al*., 2013). Nevertheless, when global dry pet food production is accessed for its environmental footprint, the results were shocking. The annual land use for dry pet food is twice the size of UK land area, and if the industry is a country, it would be the 60th biggest greenhouse gas emitter in the world (Alexander *et al*., 2020).

When Alexander *et al*. (2020) rated the environmental impact per kg of dry pet food by types, it became clear that the protein ingredients of pet food have the most negative effects on total environmental footprint of pet food. Meat accounts for more greenhouse gas emissions, therefore premium pet food – which contains more meat than by-products – is rated worse than the market-leading ones. It is worth noting that the authors did not count fish and fish by-products in the calculation because they did not have sufficient data on these ingredients, but it does not change the fact that proteins in pet food make significant impacts on the environment.

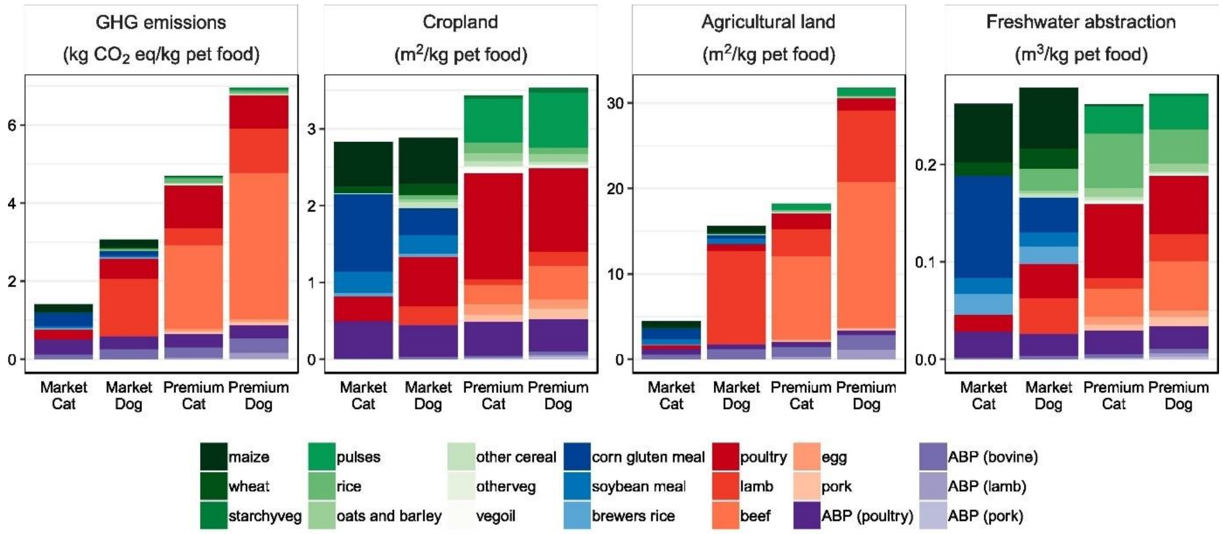

Figure 1: Rate of environmental impact per kg of by pet food type (Alexander et al., 2020)

Many studies has indicated that sustainability information has little to no effects on customers' decision to purchase pet food, and if there is, it might be because they dub environmental friendliness with the product being "animal friendly" (Kwak & Cha, 2021; O'Rourke & Ringer, 2016).

In summary, the pet food industry plays a considerable role in system sustainability, but after all it is still customer demand-driven industry. For that reason, any attempt to solve the sustainability problem of pet food production must consider the consumers' acceptance.

1.3.1. The search for alternative protein ingredients

The needs for alternative protein sources exist. New protein ingredients are needed, not only to resolve the problem with pet food production's sustainability, but also to give the producers more options to formulate the unconventional diets.

A lot of research is conducted on the application of different insects in pet food, namely housefly pupae, black soldier fly larvae and pupae, cricket, mealworm, cockroaches (Bosch *et al*., 2014; Bosch & Swanson, 2020; Van der Spiegel *et al*., 2013); they identified insects as a safe and good-quality protein source for dog and cat foods. The only concerns with insects are contamination (mycotoxins, natural toxins, heavy metals, veterinary residues, pesticides, and pathogens) from the insects' feed, and the insects themselves might be allergens or carrying pathogens. Nevertheless, insects are now listed in the Catalogue of feed materials (published by European Parliament and the Council).

Several other alternative ingredients have been researched and proposed as the non-animal, sustainable protein sources, such as microbial biomass (Sørensen *et al*., 2011), yeast (Agboola *et al*., 2021), microalgae and seaweed (Gong *et al*., 2018; Valente *et al*., 2019; Van der Spiegel *et al*., 2013). To be a protein ingredient for animal feed production, first it must have a desirable nutritional quality, such as relatively high protein content, good amino acids profile, high digestibility, good palatability (Gatlin III *et al*., 2007). Its effects on physical characteristics and heat tolerance must also be taken into consideration because they affect final product quality. Other factors should also be considered are its performance during processing, its commercial availability and price, as well as handling, shipping, and storage conditions.

It is not easy to answer mentioned questions, but we should not be discouraged from looking for new protein sources.

1.3.2. Novel ingredients used in the thesis.

Among types of animal feeds (based on target animals), aquaculture feed, especially salmon feeds, and pet food have a lot in common. Firstly, they both use extrusion technology for production. Secondly, the target animals are both omnivores (tilapias and dogs) and strict carnivores (salmons and cats). Therefore, novel ingredients that have been researched for aquaculture should also be investigated for its application in pet food as well. Historically, insects were also researched in aquafeeds application before being experimented in pet food and made it into commercial products.

Microalgae *Desmodesmus* sp. and vital wheat gluten are being experimented for replacing fish meal in fish feeds with positive results. Potato protein is also a possibility, although more research on its application is needed with regard for methionine content. This thesis investigates their potential to replace poultry meal in extruded dry pet food and discusses their effects on physical properties of extruded dry pet food.

1.3.2.1. Microalgae and the potential of Desmodesmus sp.

Biofuels production is predicted to increase further in the next decade. It is expected that biofuels should meet around 5.4% of road transport energy demand in 2025, rising from just under 4.8% in 2019; even though COVID-19 has reduced 11.6% of total biofuel output in 2020 – the first contraction in the last two decades (*Renewables 2020*, 2020). Among the third-generation biofuels feedstocks, microalgae is deemed to be "only fuel source that can be sustainably developed in the near future" (Ramaraj *et al*., 2015), which provides more oil per kg biomass than the best oil crops (Behera *et al*., 2015). Interestingly, the high amount of lipid is not the only valuable component of microalgae, their defatted biomass after lipid extraction is also a great animal feed ingredient with the potential to replace other protein ingredients such as fish meal in aquafeed (Gong *et al*., 2018; Valente *et al*., 2019), or corn and soybean meal in swine and poultry diets (Gatrell *et al*., 2014). However, there is a lack of research on the application of defatted microalgae biomass in pet food products.

The *Desmodesmus* sp. defatted biomass (DGM), which is a by-product after lipid extraction, is a potential protein-rich ingredient for animal feed. Depends on the species and the processing technology, DGM may have protein content of 20.6 % - 38.2%, and its fat content varies greatly 1.5% - 10% (Ekmay *et al*., 2014; Gong *et al*., 2018; Manor *et al*., 2017). Like other microalgae, research on DGM applications in pet food production or its effect on physical quality of extruded product is scarce.

1.3.2.2. Vital wheat gluten

According to the Catalogue of feed materials, vital wheat gluten is defined as "wheat protein characterized by high viscoelasticity as hydrated, with minimum 80 % protein (N $\times$ 6.25) and maximum 2 % ash on dry substance." It is primarily used as a gluten fortifier to improve dough structures of baked products.

Just like other plant proteins, wheat gluten is a good binder. It also improves dough elasticity (Day *et al*., 2006) and pellet texture (Draganovic *et al*., 2011; Ghorpade *et al*., 1997). When wheat gluten replaces fish meal in a fish feed diet, it increases expansion and gives the extruded product a smooth surface and a more compact structure (Draganovic *et al*., 2013), which is desirable for pet food.

Wheat gluten is regarded as a protein with high nitrogen digestibility (Apper-Bossard *et al*., 2013), although lysine content is limited (Gatlin III *et al*., 2007). It has low fat and moisture content, but it can bind fat and water well while increasing protein content of the product (Day *et al*., 2006). Inclusion of wheat gluten in extruded diets increase their cohesiveness, springiness and cutting strength (Ryu, 2020). These characteristics make wheat gluten a promising alternative protein source for extruded pet food (both dry food and chewy treats), and for "meaty" pieces in moist pet food.

1.3.2.3. Potato protein

Potato protein is a by-product from potato starch processing plants. Potato protein products typically contains about 75% protein of total weight.

Potato protein that are not heat-treated contains high levels of proteolytic enzymes inhibitors and glycoalkaloids (Wojnowska *et al*., 1982), which reduces feed intakes and thus, fish growth. This might have been the reason that many researches on using potato protein in fish feed also resulted in reduced fish growth (Xie, S & Jokumsen, A, 1997), (Xie, S & Jokumsen, Alfred, 1997). It can be concluded that only potato protein that is low in solanidine glycoalkaloids should be used in feed production, as it does not have negative effects on the animal (Refstie & Tiekstra, 2003).

In contrast to wheat gluten, limited research has been conducted on potato protein's effects on extruded products and their physical qualities.

1.4. Extrusion technology, and factors that influence pellet physical qualities.

Extrusion is the process that compresses the material and force it through a die; during which the original material undergoes increasing temperature, pressure, and shear. This results in changes in physical and chemical properties of original material.

Extrusion gives manufacturers a lot of flexibility for one production line. Depending on the ingredients, processing parameters and die design, a wide range of products with different characteristics can be produced using the same extruder. Extrusion can process various ingredients from all sources, operates continuously and lowers production cost (compared to other processing methods) (Riaz & Rokey, 2011). Currently, extrusion is the primary processing method in pet food production. About 95% pet food (for dogs and cats) in the world is produced by extrusion (Spears & Fahey Jr, 2004).

There are three ranges of factors to manipulate the extrusion process and characteristics of extruded products: product formulation, equipment selection and some other factors. Each of them is briefly discussed in section 1.4.1, 1.4.2 and 1.4.3.

1.4.1. Effects of ingredients on extrusion process and extruded pellets

Starch, protein, fat, and fiber have different influence on physical characteristics of extrudate. Starch is a better binder than other ingredients, and often improves expansion, texture, and durability of pellets. Plant protein also has positive effects on physical properties of extruded pellets, although its effects are not as great as starch. Animal proteins' functions are usually weaker than those of plant protein, or they do not have any effects at all. The effects of starch, protein and lipids on extruded products are discussed in section 1.4.1.1, 1.4.1.2 and 1.4.1.3.

Typically, dry cat food and dry dog food contains very little fiber (mostly < 6%). At this level, the effects of fiber on extruded product is minimum (Riaz & Rokey, 2011).

In general, fat-soluble vitamins are considered quite stable during extrusion, with loss rate about 15%-20%. Water-soluble vitamins, like vitamin B and C, are much less stable (Table 2). Generally, the higher moisture level of the diet, the less vitamin losses.

Table 2: Loss rate of vitamin B and C during extrusion (Frame & Harper, 1994)

Vitamin	Loss rate
Vitamin C	0% - 87%
Vitamin B1	6% - 62%
Vitamin B2	0% – 40%
Vitamin B6	4% - 40%
Vitamin B12	1% - 40%
Vitamin B3 (Niacin)	0% - 40%

Common solutions for vitamin losses during extrusion are 1) using more vitamins than needed to compensate for the loss, 2) using encapsulated form of vitamins, which is more heat-stable, and 3) including vitamins in the coating mixture (which is applied on extruded pellets).

1.4.1.1. Starch

Starch is a cheap and efficient energy source in pet food. Typically, cat food contains minimum 30% starch of total weight, and dry dog food contains minimum 40% starch (Frame & Harper, 1994; Rokey *et al*., 2010). Common starchy ingredients used in pet food formulations are cereals, oil seed meals and legumes. In grain-free formulations, tubers and roots are used instead of cereals.

Dogs and cats cannot digest raw starch well, therefore the starch must be gelatinized during processing. Starch absorbs water during gelatinization, becomes soluble and binds ingredients. It also improves expansion and contributes to forming extrudate's porous texture. In other word, only gelatinized starch has influence on extrudate texture.

Starch requires sufficient moisture and energy to achieve gelatinization. Because of this, higher energy and higher moisture during extrusion improves starch gelatinization. The energy can come from both heat and shear.

Increasing shear (by changing screw configuration or screw speed) improves expansion, but this is not always the case for moisture. Higher moisture, while improving gelatinization, decreases shear thus reduces expansion. However, if there is not enough water to gelatinize starch, expansion is also reduced.

Different sources of starch with different compositions and grain structures perform differently during extrusion. Higher amylose content in the diet results in increased expansion. Hard grains (hard wheat, barley,…) hydrate slowly, therefore they should be ground before extrusion. Potato and tapioca starch may not contribute to expansion as much as high-amylose corn starch, but they often give the extruded pellets smoother surfaces.

1.4.1.2. Protein

Protein is not only essential for animals, but also has many other functions in the diet. Generally, most proteins absorb water in the mash mix and bind ingredients together. Most proteins in pet food formulation are either plant-based or animal-based. Plant proteins are cheaper, but animal-based protein ingredients make the final product more palatable.

Often plant protein gives better extrudate structure than animal protein, because they usually do not go through high heat processing. Therefore, they are included in various extruded products, from dry pet food to "meaty" chunks in wet food. Most plant proteins in pet food comes from proteinaceous ingredients like corn gluten meal. wheat gluten, soybean flour and meal. Cereals and legumes, such as whole wheat, whole grain corn, peas also contain a decent amount of protein (10% - 15%, depending on the variety).

Common animal proteins used in pet food are fish meal, meat meal and other slaughterhouse by-products like blood meal or feather meal. They are usually prepared at high temperature to be microbiologically safe and, consequently, are denatured. For this reason, animal proteins' functions tend to be weaker, or do not support extrudate structure at all.

Protein's effects on the physical qualities of pellets are weaker than those of starch. Most of the time, increasing protein content of a formulation in expense of starch results in weaker pellet durability, and firmer and/or harder pellet texture.

1.4.1.3. Lipids and fibers

Pet food usually contains minimum fat content of 9% for cat food and of 10% for dog food. Fats and oils are important energy and essential fatty acid source, and a carrier for fat-soluble components such as vitamins. Lipids in pet food formulation either comes from plant sources (vegetable oils), or animal-based ingredients such as poultry fat, beef fat, fish oil,… Fish meal and other meat meals also contain certain amount of lipids.

Fats and oils reduce friction between particles, as well as between the feed mix and the components of the extruder. For this reason, higher fat inclusion in the formulation reduces expansion and increases bulk density of extruded pellets. Fats and oils can be added directly into the extruded diet or are applied on extruded pellets by coating.

A general rule for effects of fat inclusion in pet food formulation is shown in Table 3.

Table 3: Effects of fat inclusion level on physical characteristics of extruded dry pet food, according to Riaz and Rokey (2011)

Fat inclusion level	Effects on physical characteristics
< 7%	Not much effects on physical qualities of extruded pellets.
7% - 12%	Expansion is reduced, and product density increases.
12% - 17%	Expansion is reduced significantly, pellet durability may or may not reduce.
> 17%	Pellet durability may reduce significantly.

1.4.2. Effects of process design, equipment selections and configurations

1.4.2.1. Process design

Before going into extruder, the feed diet may be pre-conditioned using pre-conditioner. During pre-conditioning, the materials are mixed with steam or hot water, and retained in the pre-conditioner for a period before extrusion. Preconditioning hydrates ingredients, which improves gelatinization during extrusion and reduces extruder barrel wear (Frame & Harper, 1994). Preconditioning is an optional step in dry pet food processes.

1.4.2.2. Single-screw extruders vs. twin-screw extruders.

Both single-screw and twin-screw extruders are used in dry pet food production. Each of them has their own pros and cons, as well as different applications (Table 4). Twin-screw extruders are more expensive and requires higher maintenance and operation cost. Therefore, they are more widely used for high-value products to justify the costs.

Table 4: Comparison between single-screw extruders vs. twin-screw extruders, according to Frame and Harper (1994) and Riaz and Rokey (2011)

	Single-screw extruders	**Twin-screw extruders**
Application	Low-fat products (ideally <8%). Low-moisture formulations.	High-fat products (> 17%). Very sticky, oily, viscous formulations, or those that require up to 30% meat and/or other high moisture ingredients. Co-extruded products. Also used in small-scale plants, that produces various products with vastly different formulations, shapes, and sizes.
Other advantages	Easy to assemble screw configurations (compared to twin-screw extruder).	Self-cleaning (only extruders with intermeshing screws).

1.4.2.3. Equipment configurations

Die orifice design not only affects size and shape of the extruded products, but also have significant effects on mechanical energy, degree of starch gelatinization and pellet appearance. Figure 2 (from (Frame & Harper, 1994)) shows results of an extrusion experiment, which only changed the die design. Compared to the normal circular die (inlet size = outlet size), the tapered circular die (larger inlet and smaller outlet) results in higher mechanical load, but poorer starch gelatinization and different shape.

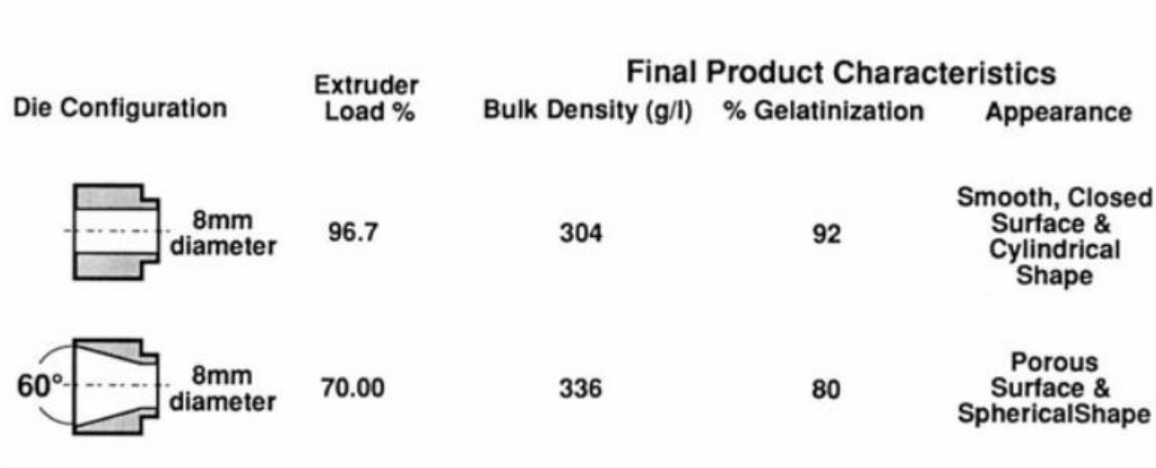

Figure 2: Effects of die orifice design on pellets' characteristics, from (Frame & Harper, 1994)

Different shapes and sizes of screw elements play different roles during extrusion. There are many types of screw, including but not limited to single and double flight screws, cut and folded screw, shallow and deep flight screws, shear rings (pentagon),… (figure 3 - 5) To put it simply, the screw elements are assembled to transport the materials from the feeder to the die, while creating sufficient shear to cook the materials.

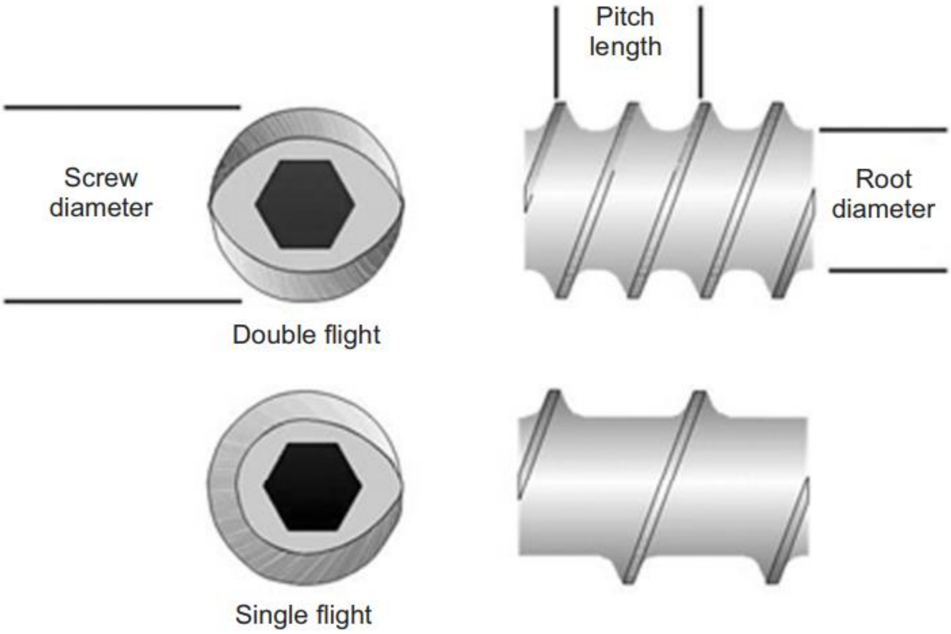

Figure 3: Single flight screw and double flight screw, from (Riaz & Rokey, 2011)

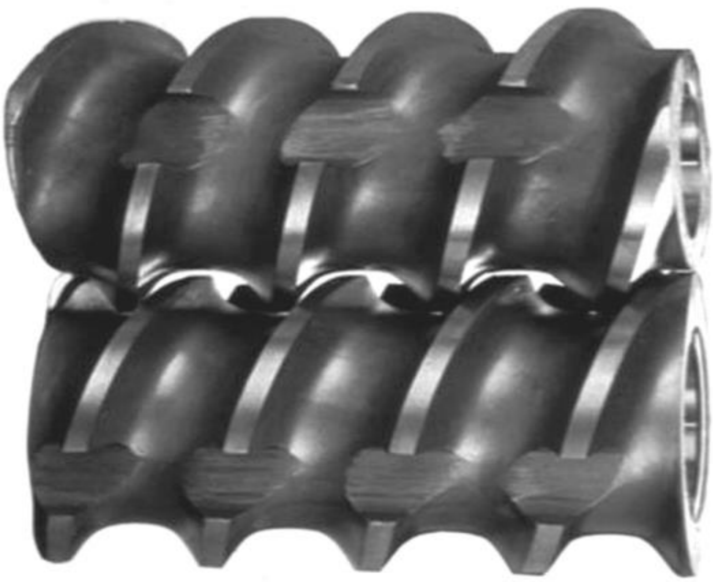

Figure 4: Cut flight screws, from (Riaz & Rokey, 2011)

Figure 5: Shear rings (pentagons), from (Riaz & Rokey, 2011)

1.4.3. Other factors

The raw ingredients are usually different in size, some might be agglomerated during storage. Although extruders can handle a range of particle size, grinding materials to the proper particle sizes has many benefits:

- Proper particle size = better pellet appearance. If the materials are ground and mixed properly before extrusion, the product will less likely have different fragments on different particles.
- The outlet is less likely to be plugged.
- Improving cooking efficiency as well as pellet structure.

As a rule, all particle sizes of materials should be less than one third of the orifice diameter, but no particle should be bigger than 1.5 mm (Riaz & Rokey, 2011).

In extrusion context, rework includes fines, broken products, and under-processed materials. Rework can be dried, ground and added into the recipe at 5% - 10% level (Riaz & Rokey, 2011). However, the addition should be consistent throughout production. In consistent addition results in inconsistent products as rework has very week binding capacity and usually has different colors to other ingredients.

1.5. Physical characteristics of extruded pet food

For dry pet food, physical qualities are just as important as nutritional characteristics. The physical qualities are critical to the stability of the product during storage (BAŞER &

Yalcin, 2017), therefore the effects of an ingredient on the physical qualities of the final products should be carefully evaluated before large-scale production.

Water activity has great influence on product shelf-life. Water activity is defined as the ratio of the vapor pressure of a sample and the vapor pressure of pure water at a given temperature, while moisture is the amount of water in the sample. Water activity level under 0.6 inhibit the microbial growth, while the moisture level may or may not correlate with the durability of the pellet (BAŞER & Yalcin, 2017). If the product is expected to have water activity > 0.6, humectants and mold inhibitors must be used to prolong shelf-life.

Durability determines the physical stability of the product during storage, handling, and transportation. If the durability of the pellet is low, the product will be broken, or even crumbled before it reaches the customers.

Beside mentioned characteristics, expansion ratio and hardness are also taken into consideration. The measurement and effects of these qualities on the final products will be discussed in the Discussion as well as Material and Methods sections.

1.6. Aim and objectives of the thesis.

Although research have been conducted on application of microalgae, vital wheat gluten and potato protein in extruded feeds (Apper-Bossard *et al*., 2013; Gong *et al*., 2018; Xie, S & Jokumsen, Alfred, 1997), there are limited understanding on how these ingredients affect the physical qualities of extruded feed pellets, or how they perform in a pet food diet. The aim of the thesis is to gain more understanding on how the mentioned ingredients change physical qualities of extruded dry pet food. Characterization of functional physical characteristics of pellets produced with microalgae, vital wheat gluten and potato protein are conducted to gain more insights on the effects of these ingredients. Result obtained from the experiments would contribute to a better understanding how microalgae and the plant proteins perform in an extruded feed recipe, and it would be useful in research and developing extruded products that include them.

Typically, defatted microalgae biomass has much lower protein content compared to poultry meal, vital wheat gluten or potato protein. Therefore, it would be useful to study it separately. Regarding this matter, and to achieve the aim of thesis, the objectives of the thesis are:

1) Investigate the effects of defatted microalgae biomass on physical qualities of extruded pellets produced with microalgae at different inclusion rate in a dog food recipe.
2) Investigate the effects of replacing poultry meal with vital wheat gluten and potato protein in a 1:1 ratio on physical qualities of extruded pellets.

To achieve the objective 1), experiment 1 was conducted, in which poultry meal was partially replaced with DGM at different inclusion levels (10%, 15%, 20%) in an extruded dog food recipe. Pellets produced from these diets were subjected to physical quality analyses. The results were then used to investigate of how DGM affect the physical qualities of pet food at different rates.

Experiment 2 was carried out to achieve objective 2), in which poultry meal is replaced with vital wheat gluten and potato protein at ratio 1:1, in an extruded pet food recipe. Pellets produced with these ingredients were subjected to physical quality analyses. The results were then used to investigate of how the mentioned plant-based protein affect the physical qualities of pet food at different rates.

The results from two experiments were then discussed further to gain more insights about effects of these ingredients, regarding physical qualities of extruded feeds.

2. Material and Methods

2.1. Ingredients and formulation

Six types of experimental feeds were produced at Center for Feed Technology (Fôrtek) at Norwegian University of Life Sciences. Information about their ingredients is noted in Table 5.

The pre-extrusion mixture of the control dog food diet (diet 1) contained 37.5% of poultry meal by weight. For experiment 1, diets 2, diet 3 and diet 4 were formulated, in which poultry meal was partially replaced with DMG at inclusion rate 10%, 15% and 20% of the total pre-extrusion mixture, respectively. For experiment 2, diets 5 and diet 6 was formulated, in which poultry meal was replaced with the same amount of vital wheat gluten (WP) and potato protein (PT), respectively. The formulation of all experimental diets aims to similar proximate composition. The recipes and proximate composition of the experimental feeds are included in Table 5.

Table 5: The recipes and proximate composition of the experimental feeds

Feed ingredients (g/100 g pre-extrusion mixture)	**1** Control diet	**2** 10% DMG	**3** 15% DMG	**4** 20% DMG	**5** WP	**6** PT
Poultry meal[1]	37.5	30	25	20		
Microalgae (DMG)[2]		10	15	20		
Vital wheat gluten (WP)[3]					37.5	
Potato protein (PT)[4]						37.5
Wheat flour[5]	22.5	22.5	22.5	22.5	22.5	22.5
Rapeseed meal[6]	15	15	15	15	15	15
Beet pulp[7]	9	6.5	6.5	6.5	13	13
Soybean oil[8]	8	8	8	8	4	4
Guar Gum[9]	5	5	5	5	5	5
Glycerol[10]	2	2	2	2	2	2
Monocalcium phosphate (MCP)[11]	1	1	1	1	1	1
Proximate composition (g/100 g pre-extrusion mixture)						
Crude Fiber	6.24	5.98	6.01	7.18	4.98	4.77
Crude Protein*	34.51	34.34	31.86	30.01	40.71	41.53
Starch	13.98	15.12	15.08	16.67	17.97	11.35
Crude fat	8.87	7.96	8.48	6.68	2.52	3.24

[1] Norsk protein AS, Norway

[2] Cellana, Hawaii – USA

[3] Roquette Amilina, Lithuania

[4] Cargill, Denmark

[5] Møllerens Siktet Hvetemel, produced by Møllerens, Norway.

[6] Felleskjøpet, Norway

[7] Felleskjøpet, Norway

[8] DENOFA AS, Norway

[9] Felleskjøpet, Norway

[10] Bergen Engros AS, Norway

[11] Yara Animal Nutrition

*The protein content is calculated using the nitrogen-to-protein conversion factor of N x 6.25.

2.2. Feed production process

The entire production process, from ingredients preparation to final products, was done at Fôrtek. All diets are produced using the same equipment and screw configuration. The feeds were not pre-conditioned before extrusion. A simplified process flowchart of experimental feed production is shown in Figure 6.

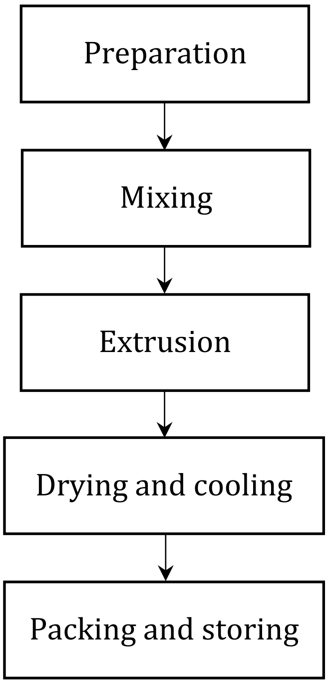

Figure 6: Simplified process flowchart of experimental feed production

- **Preparation**: this step is to ensure for homogenous mixing of the process, including

  - Sieving and grinding DMG, as it is prone to clumping. DMG was sieved manually. The clumps are grounded in a hammer mill to ensure the final particle size is < 1 mm.
  - Weighing ingredients to ensure the right amount was used. Each diet is prepared in a batch of 70kg.
- **Mixing:** this step is conducted in a small 60L mixer at Fôrtek. All ingredients were mixed until becoming a homogenous mixture.
- **Extrusion:** a twin-screw co-rotating extruder Bühler BCTG 62 was used, Bühler BCTG 62 The feed mix was added into the feeder manually because the production was conducted for small patches.
  - Screw configuration used in the experiments are shown in Table 6 . The screw configuration is the same for all diet. The configuration was done by Fortek to optimize the production, I did not participate in this process because of Covid-19 restriction. Information regarding screw configuration was provided by Fortek.
  - Sample pellets were collected when the process parameters are stable.
  - Extrusion parameters:
    - The feeds are produced at similar parameters, except slight differences in screw speed and extrusion water for production of diet 3 (15% DMG) and diet 4 (20% DMG). This change was to improve binding for the pellets – it was made by Fortek personnel, and it was an effective choice. Effects of this change is discussed further in 3. Results and discussion.
    - Extrusion water for diet 5 and 6 are higher than other diets, because plant-based ingredients usually contain more starch than poultry meal, therefore it is reasonable to use more water to improve hydration and gelatinization of starch.
    - All extrusion parameter is shown in Table 7.

Table 6: Screw configuration used in the experiments.*

(front)							90°	90°	R					R					**Length**
40	40	60	60	60	80	80	20	20	120	100	80	60	40	60	100	80	80	80	**1260**
R	R	R	R	R	R	R	L	L	POLY	R	R	R	R	POLY	R	R UC	R UC	R	(feeder)

**L, R and UC stands for left-turned, right-turned, and undercut elements, respectively. The red arrows are where the spacers are (in the extruder barrel). The unit of measurement is mm.*

Table 7: Extrusion parameters during production of experimental feeds

	1	2	3	4	5	6
	Control diet	**10% DMG**	**15% DMG**	**20% DMG**	**Vital wheat gluten**	**Potato protein**
Die size. (Diameter, mm)	7	7	7	7	7	7
Number of dies	2	2	2	2	2	2
Screw speed (rpm)	500	500	450	450	500	475
Extrusion water (kg/h)	14	14	16	16	20.5	24
Knife speed (rpm)	300	300	350	350	350	300
Number of knifes	6	6	6	6	6	6

2.3. Pellet analysis

The physical properties that are measured and analyzed are: sectional expansion index, moisture, water activity, hardness, and durability. Pellets from all types of feeds are analyzed using the same methods.

All pellets are cylinder-shaped.

2.3.1. Expansion ratio

Expansion ratio is calculated to evaluate how much the formulation expands radially during extrusion.

There was variation in diameters of pellets from the same diet, therefore a big sample size was obtained. Diameters of 130 random pellets from each diet are measured by a digital micrometer. Their expansion ratio is calculated as the ratio of the diameters of extruded pellets to the die opening (7 mm), as it was done by Fan *et al*. (1996).

2.3.2. Moisture

Moisture content (wet basis) is the percentage of water contained in the extruded pellets. Moisture content analysis was done three times for each diet. The results were then averaged.

About 15g – 16g of pellets from each diet was ground by mortar and pestle and weighed for wet weight. Then the samples were spread onto a foil dish and were dried at 105^{0}C for 10 hours. After drying, the samples were weighed again for dry weight. The moisture content was calculated using the formula:

$$Moisture\ content\ (\%) = \frac{m_1 - m_2}{m_1}$$

Where m_1 is the wet weight of the sample (before drying), and m_2 is the dry weight (after drying).

2.3.3. Water activity

Water activity is a dimensionless parameter. It is defined as the ratio of the vapor pressure of water in a sample, in an undisturbed environment, and the vapor pressure of distilled water, at the same temperature. Therefore, water activity analyses of each diet were done consecutively to prevent variation in results because of temperature change. The results were then averaged.

Water activity analysis was done three times for each diet.

Water activity of the pellets was analyzed by water activity indicator Rotronic HygroLab. The sample cups were filled two-thirds full of roughly chopped pellets. The results were obtained after 20 minutes (when the values were stable).

2.3.4. Hardness

Hardness is the force needed to break a pellet. Pellet hardness was measured by using KAHL hardness tester (the manually operated design), the unit of measurement is kg.

A pellet is placed between two bars, then the screw is tightened until the pellet is broken. The indicator marks how much force needed to break the pellet (in kg), this is the hardness value of the pellet. There was a variation in hardness, so 60 samples from each diet were tested.

2.3.5. Durability

Pellet durability is the amount of dust that will be produced after subjecting pellets to mechanical forces (Thomas & Van der Poel, 1996). Holmen NPH200 – Automatic durability tester (TEKPRO) was to test pellet durability. Pellet diameter is set to 7 mm, 100 g of pellets from each diet was used for each test.

Holmen tester records pellet durability index (PDI), which is calculated as the percentage of pellets that remains after being subjected pneumatic agitation. Durability analysis was done three times for each diet, the results were then averaged.

2.4. Data analysis

SPSS offers two tests for normality: Shapiro-Wilk test and Kolmogorov-Smirnov test. Kolmogorov-Smirnov test is a common choice for $n \geq 50$, but it is cautioned against because of low power compare to other normality tests (Ghasemi & Zahediasl, 2012). Shapiro-Wilk test is more preferred, because it can handle any sample size n in the range $3 \leq n \leq 5000$ (Royston, 1995). Therefore, Shapiro-Wilk test is used to check normality of data in the thesis.

For most parameters (except hardness and expansion ratio), results were analyzed for normality using Shapiro-Wilk test. The data was all normally distributed; therefore t-test was used to determine significant differences. Shapiro-Wilk test and t-test were carried out using SPSS.

For hardness and expansion ratio, first the data sets are subjected to the Shapiro-Wilk test for normality. Because the data was not normally distributed, medians were compared across groups (diets). Normality test and median comparation were carried out using SPSS.

3. Results and discussion

The variables during production are shown in Table 8. Each diet, combined with processing parameters, has different effects on the extrusion process.

In general, the production of pellets from all experimental diets results in higher energy consumption (which was indicated by higher drive power, SME) compared to the control diet.

Table 8: Extrusion process variables recorded during pellets production.

	1	2	3	4	5	6
Pressure, barrel 4 (bar)	0.13	0.19	0.64	0.62	0.28	0.65
SME (W·h/kg)	584	635	565.5	643	471	411
Drive power (kW)	8.2	9.1	9	10.1	9.7	9.9
Torque (%)	38	40	44	49	42	45

Diets with higher inclusion of DMG consumes more energy than the control diet. Moreover, higher SME may indicate diets that cause higher wear rates to extruder barrel components (Riaz & Rokey, 2011). More research is needed to optimize energy consumptions during production these diets, maybe by increasing extrusion water.

Production of pellets from diet 5 and 6 requires more energy than those from control diet, although the energy consumption and SME is still lower than diets contained DMG. This might also because the extrusion water rate in these diets is higher than other. Lower SME indicates producing these diets cause lower wear rate than diets from control diet and diet 2, 3, and 4.

3.1. Experiment 1: effects of the partial replacement of poultry meal with defatted microalgae biomass

Experiment 1 studied the effects of DMG on physical quality of extruded pellets when it is used to replace poultry meal partially.

Results regarding expansion ratio, water activity, moisture, hardness, and durability is shown in Table 9 (diet 1, 2, 3, 4). Statistical analysis showed that the difference in physical qualities of pellets from different diets are significant ($p < 0.05$).

Table 9: Expansion ratio, water activity, moisture, hardness, and durability of extruded pellets from diet 1, 2, 3, 4

Diet	1	2	3	4
	Control diet	**10% DMG**	**15% DMG**	**20% DMG**
Expansion ratio*	1.0529	1.04215	1.0057	1.0214
Water activity	0.592 ± 0.0021	0.624 ± 0.0012	0.614 ± 0.0006	0.582 ± 0.0012
Moisture (%)	10.947 ± 0.1549	12.496 ± 0.2489	11.978 ± 0.0621	11.679 ± 0.1501
Hardness (kg)*	8.75	9.5	9.5	11
Durability (%)	93.43 ± 0.058	96.63 ± 0.115	96.46 ± 0.058	98.4 ± 0.1

**Expansion ratio and hardness data are not normally distributed, therefore only medians are shown here.*

Effects of partial replacement of poultry meal (with DMG) on expansion ratio of extruded pellets are shown in Figure 7. Results (Table 9) showed that the increasing inclusion of DMG decrease expansion. From Table 8, it is worth noted that the control diet and diet 2 (10% DMG) share the same processing parameter (14 kg water/h, screw speed 500 rpm), but diet 2 expanded less than diet 1 significantly. To counter this effect, more water was added (16 kg/h) and screw speed was decreased (450 rpm) for diet 3 and 4. Under these conditions, diet 4 (20% DMG) expanded better than diet 3 (15% DMG), although both expanded less than the control diet.

The reduced expansion can be explained that the microalgae starch is not as easy to process as starch in cereal (wheat), and they need more water and time to hydrate and gelatinize. This also explains why pellets in diet 3 and 4, when given more water and more retention time (reduced screw speed), improve their expansion ratio. This was also observed in other studies, when high-starch diet was not given enough water or time to process, expansion is significantly decreased (Badrie & Mellowes, 1991; Jin *et al.*, 1995). Other studies investigates microalgae effects on expansion ratio shows vastly different results based on the microalgae species was used (Alcaraz *et al*., 2021; Uribe-Wandurraga *et al*., 2020), but none of these studies mentioned *Desmodesmus* sp.

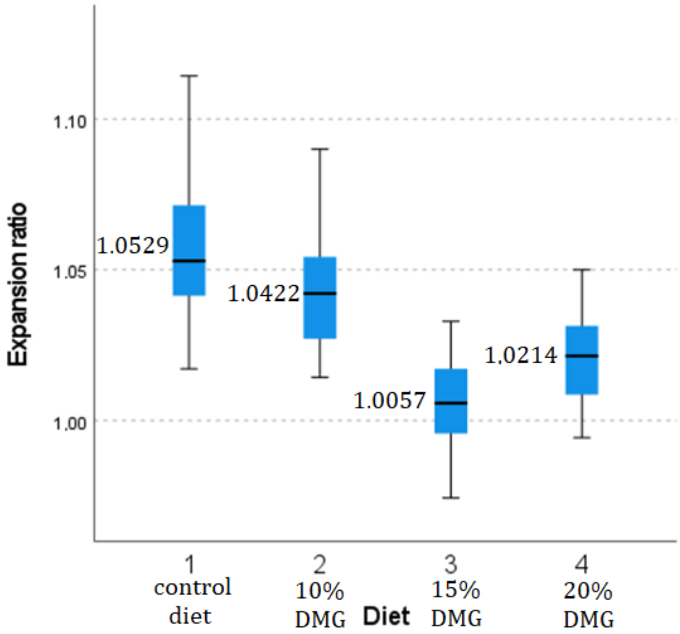

Figure 7: Expansion ratio (range and medians) of extruded pellets (from diet 1, 2, 3, 4).

Although diets made with more DMG (3, 4) showed decreased expansion ratio, it is possible that microalgae starch was not completely hydrated and gelatinized to give the best expansion ratio. More research is needed to fully explore how DMG's effects on expansion ratio in extruded diets. Possible options would be extrusion with more moisture and retention time, or with and without preconditioning, at different inclusion rates, combining with evaluation of the degree of starch gelatinization.

Effects of partial replacement of poultry meal on moisture content and water activity are shown in Figure 9, the results are shown in Table 9. All diets that include DMG contains more moisture than the control diet, thus their water activities are higher (except pellets from diet 4, higher moisture but slightly lower water activity). It is also indicated that the more DMG is included, the less moisture is contained in the pellets.

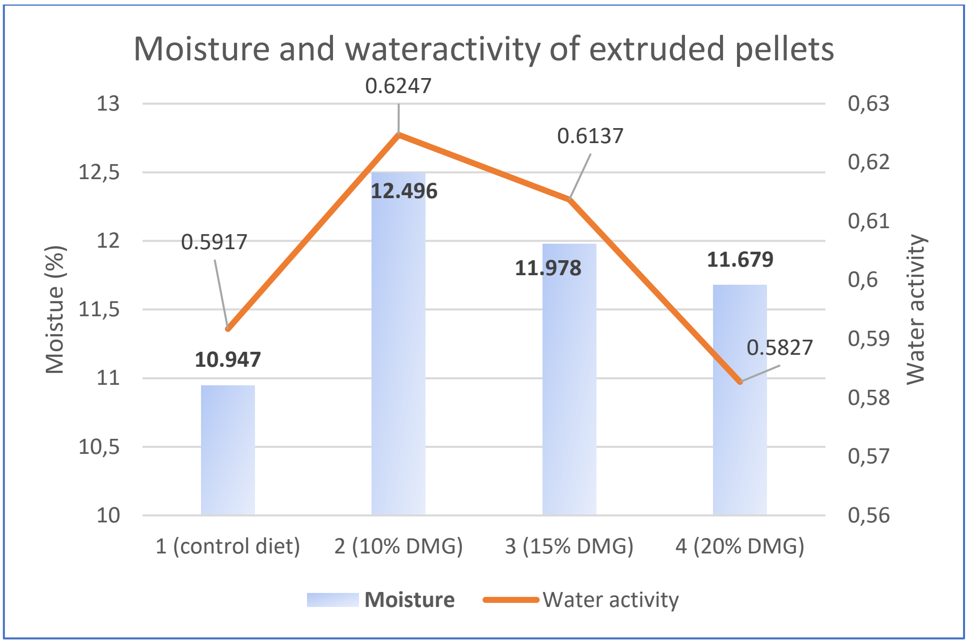

Figure 8: Moisture content and water activity of extruded pellets (from diet 1, 2, 3, 4)

Among them, only diet 4 (20%) has water activity < 0.6, and it also has lowest moisture content. Diets contain DMG has more higher moisture content and water activity than the control diet. On the other hand, higher inclusion rates of DMG leads to lower moisture content and lower water activity.

Poultry meal are usually processed at high temperature to eliminate pathogens; therefore, its proteins are denatured and do not bind water well. Meanwhile, DMG contains starch, and starch binds water better than protein. This explains why the pellets from control diet contains less moisture than pellets from diets contained DMG.

Higher DMG content results in lower moisture content and water activity. It is also worth noted what the diet 3 expands less than diet 2 and 4, but it does not contain most moisture or highest water activity. A possible explanation is that pellets from diet 2, 3, and 4 have different morphology. Pellets that contain more DMG may be more porous because it contains more starch, and their porosity aided drying efficiency. The effect of microalgae on morphology, specifically increasing pore size, is also observed by (Uribe-Wandurraga *et al.*, 2020). Nevertheless, effect of DMG on morphology of extruded pellets needs more research to investigate it better.

Effects of partial replacement of poultry meal on pellet hardness and durability are shown in Figure 9 and Figure 10, results are shown in Table 9.

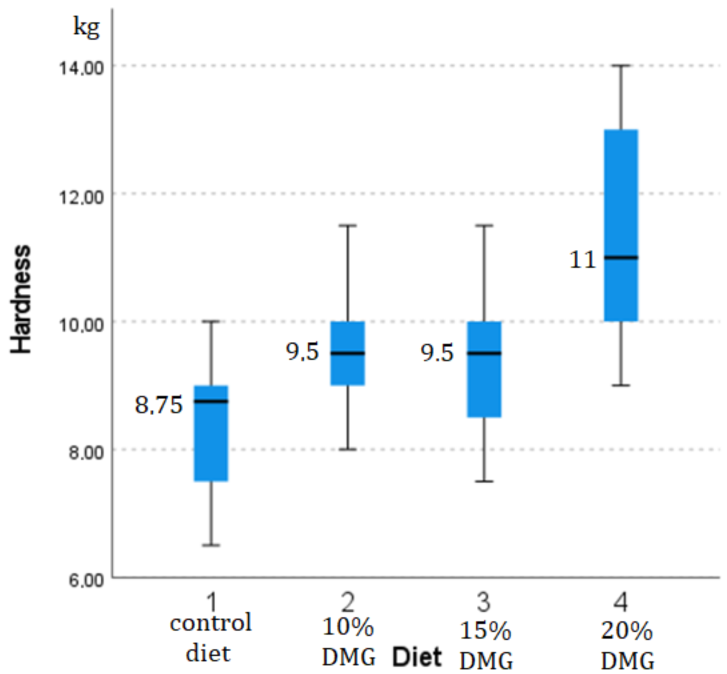

Figure 9: Kahl hardness of extruded pellets (from diet 1, 2, 3, 4)

The experiment results show that diets that include more microalgae has higher hardness and durability compared to the control diet. It is also indicated that harder pellets tend to be more durable. Pellets from diet 4 (20% DMG) has highest hardness, also has highest durability.

From the results, it can be inferred that the inclusion of microalgae increases hardness and durability of extruded pellets. Higher microalgae inclusion is usually associated with an increase in hardness and durability of extruded pellets, as observed in (Alcaraz *et al.*, 2021; Gong *et al.*, 2019), regardless microalgae species.

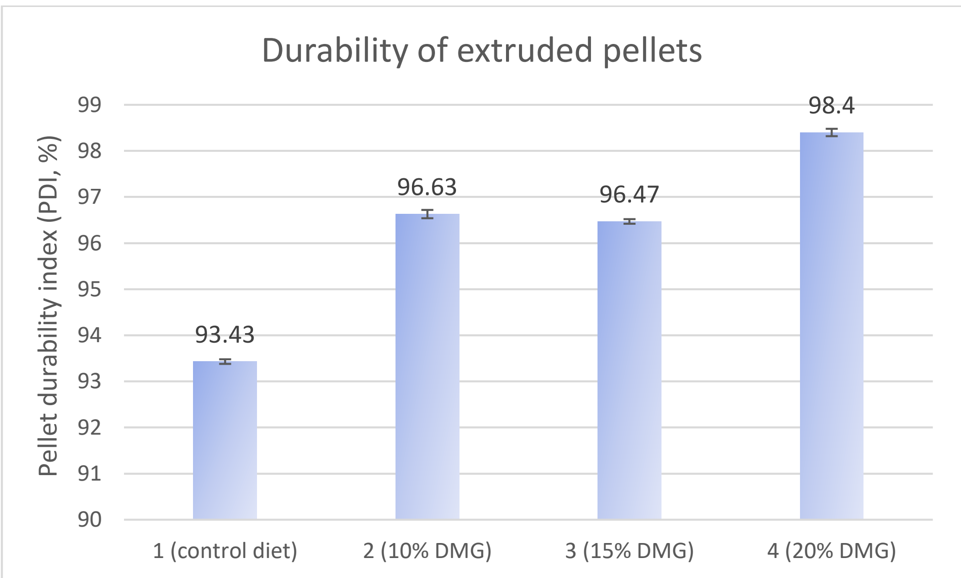

Figure 10: Durability of extruded pellets (from diet 1, 2, 3, 4)

3.2. Experiment 2: effects of the replacement of poultry meal with vital wheat gluten and potato protein

Experiment 2 investigated the effects of vital wheat gluten and potato protein when they are used to replace poultry meal completely, at ratio 1:1.

Results regarding expansion ratio, water activity, moisture, hardness, and durability is shown in Table 10. Pellets from diet 4 (20% DMG) in the first experiment have comparable water activity with pellets from control diet. Therefore, results of diet 4 from experiment 1 are included in Figure 11 – Figure 14 for comparison and discussion. Statistical analysis showed that the difference in physical qualities of pellets from different diets are significant ($p < 0.05$).

Table 10: Expansion ratio, water activity, moisture, hardness, and durability of extruded pellets from diet 1, 4, 5, 6

Diet	**1**	**4**	5	6
	Control diet	**20% DMG**	**Vital wheat gluten**	**15% DMG**
Expansion ratio*	1.0529	1.0214	1.0843	1.085
Water activity	0.592 ± 0.0021	0.582 ± 0.0012	0.617 ± 0.0006	0.688 ± 0.0015
Moisture (%)	10.947 ± 0.1549	11.679 ± 0.1501	26.694 ± 0.1659	28.1 ± 0.0711
Hardness (kg)*	8.75	11	13	10.5
Durability (%)	93.43 ± 0.058	98.4 ± 0.1	96.57 ± 0.306	98.27 ± 0.153

**Expansion ratio and hardness data are not normally distributed, therefore only medians are shown here.*

In diet 5 and 6, poultry meal was replaced completely with vital wheat gluten (WP) and potato protein (PT) at ratio 1:1. The effects of this replacement on expansion ratio are shown in Figure 11.

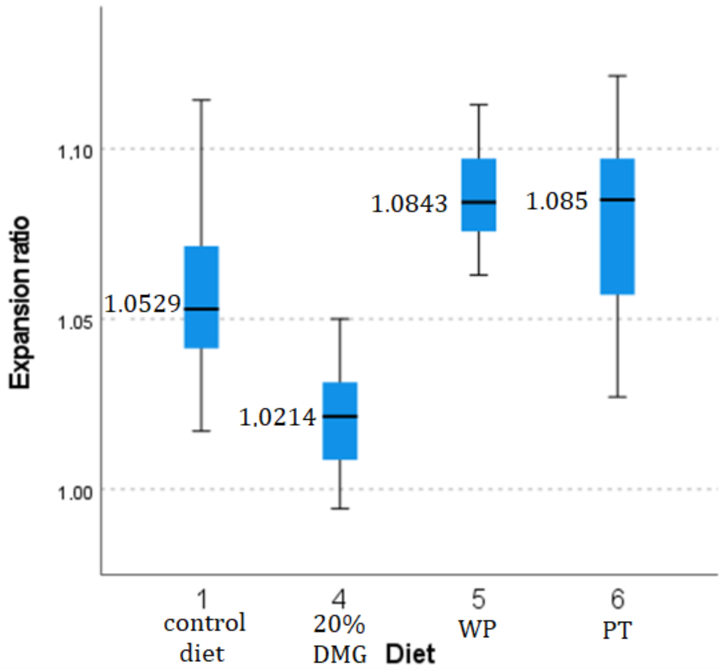

Figure 11: Expansion ratio (range and medians) of extruded pellets (from diet 1, 4, 5, 6)

Results show that replacing poultry meal with the plant proteins increases expansion. Expansion ratio of pellets from diet 5 and diet 6 are not significantly different ($p > 0.05$). Both diets produce pellets that expanded more than the control diet and diet 4 (Figure 11). This is predictable, because poultry meal is processed at high heat and denatured, therefore its functions are not as good as plant proteins. Many other studies observed the same effects from other plant proteins (Draganovic *et al.*, 2013; Sørensen *et al.*, 2009).

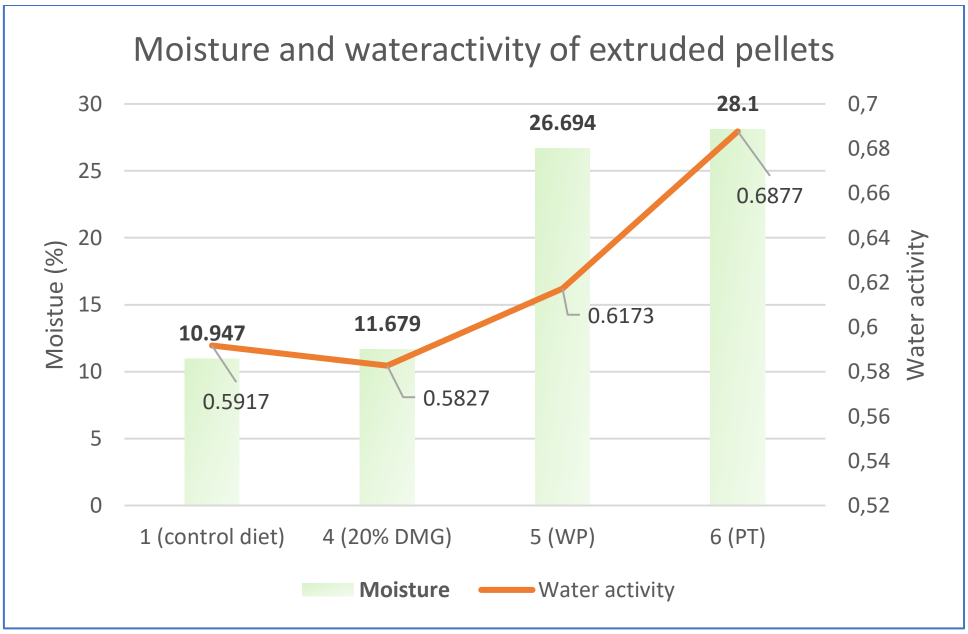

Figure 12: Moisture content and water activity of extruded (from diet 1, 4, 5, 6)

Effects of the replacement of poultry meal with vital wheat gluten and potato protein on moisture content and water activity are shown in Figure 12 Both diets 5 and 6 contain more moisture and higher water activity than the control diet. The water activity of pellets from these diets are too high (> 0.6), which would encourage mold growth.

There was little understanding on potato protein to predict anything, but it was unexpected that pellets from diet 5 had high moisture content. High wheat gluten inclusion tends to result in big pore size (Draganovic *et al*., 2013), and that should have encouraged higher moisture efficiency. It is possible that the feed was produced with high water content, and that results in the high moisture level. More research is needed to find the optimized moisture content for extruded diets using wheat gluten.

More research needs to be done to further understand the morphological effects of potato protein to draw better conclusion on the ingredients, and that should be done at many extrusion-water levels to find the optimized moisture content.

Effects of replacing poultry meal with vital wheat gluten and potato proteins on pellet hardness and durability are shown in Figure 13 and Figure 14, results are shown in Table 10.

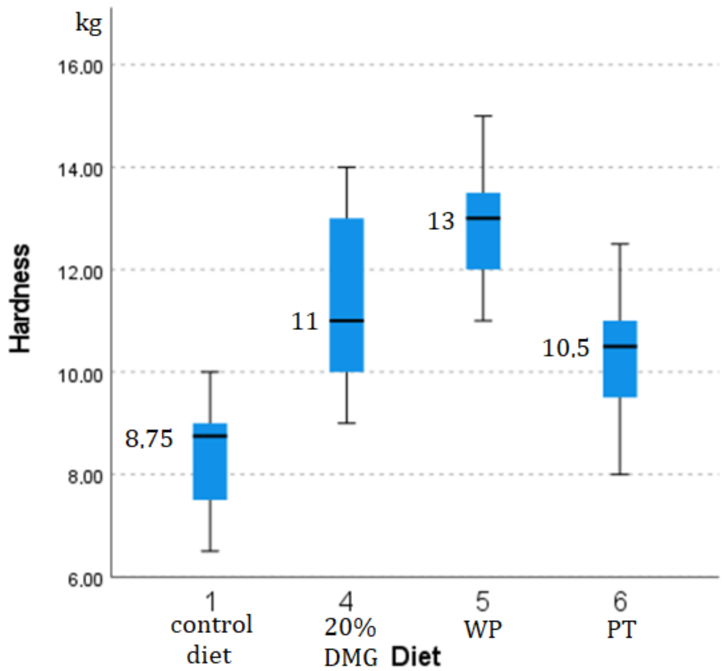

Figure 13: Kahl hardness of extruded pellets (from diet 1, 4, 5, 6)

Effects of replacement of poultry meal with plant proteins on pellet hardness and durability are shown in Figure 14 and Figure 15. Pellets made with WP and PT are harder and more durable than those from control diet. This is foreseeable, as they are more functional than poultry meal (which was denatured even before extrusion). Other studies also reported the same effects of wheat gluten (Draganovic *et al*., 2011; Draganovic *et al*., 2013). Interestingly, the effects of vital wheat gluten and potato protein on hardness and durability are not the same.

Pellets made with vital wheat gluten were the more brittle, with highest hardness but weaker durability than pellets from diet 4 and diet 6. However, this is not problematic because the pellet durability index is still high, and higher than those made from poultry protein. On the other hand, pellets from diet 6 are more durable than those from pellets 5 although not as hard. However, it is an improvement comparing to pellets from the control diets.

From the results, it can be concluded that vital wheat gluten and potato protein have different effects on hardness and durability of extruded pellets, but generally both improve pellet hardness and pellet durability index.

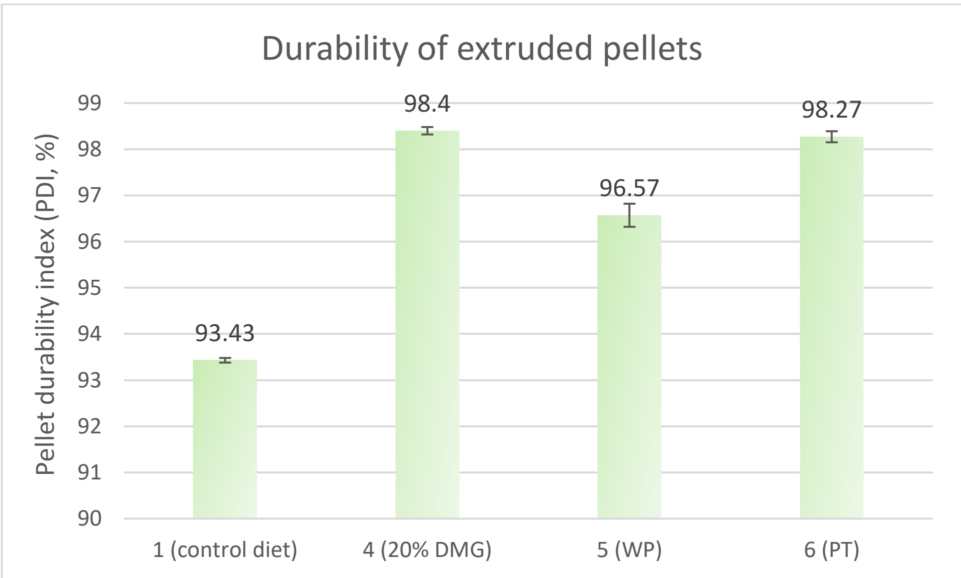

Figure 14: Durability of extruded pellets (from diet 1, 4, 5, 6)

4. Conclusion

The effects of partially replacing poultry meal with DMG showed that higher DMG inclusion did improve hardness and pellet durability, and optimal inclusion rate of DMG was 20%. However, more research is needed to understand its effects on expansion, probably by improving hydration and evaluating the degree of starch gelatinization.

The effects of replacing poultry meal with vital wheat gluten and potato protein revealed that the effects of these ingredients on physical quality are mostly similar to what have been observed in other studies. However, pellets made with these ingredients showed high water activity (> 0.6). Therefore, more research is needed to improve drying efficiency, both by better understanding their effects on pellet morphology and optimizing the extrusion water rate.

Results from this thesis shows that DMG, vital wheat gluten and potato protein has the potential to replace animal protein in extruded feeds, especially pet food, regarding physical qualities. However, more studies and experiments are needed to understand better how they perform in extruded diets to optimize formulation as well as processing parameters, without compromising certain physical traits or energy consumption.

5. References

Agboola, J. O., Øverland, M., Skrede, A. & Hansen, J. Ø. (2021). Yeast as major protein-rich ingredient in aquafeeds: a review of the implications for aquaculture production. *Reviews in Aquaculture*, 13 (2): 949-970.

Alcaraz, R., Hernandez-Contreras, A., Iglesias, P. & Hernandez, M. (2021). Effect of the inclusion of microalgae on the physical properties of extruded feed for gilthead seabream (Sparus aurata L.). *Algal Research*, 53: 102167.

Alexander, P., Berri, A., Moran, D., Reay, D. & Rounsevell, M. D. (2020). The global environmental paw print of pet food. *Global Environmental Change*, 65: 102153.

Apper-Bossard, E., Feneuil, A., Wagner, A. & Respondek, F. (2013). Use of vital wheat gluten in aquaculture feeds. *Aquatic biosystems*, 9 (1): 1-13.

Axelsson, E., Ratnakumar, A., Arendt, M.-L., Maqbool, K., Webster, M. T., Perloski, M., Liberg, O., Arnemo, J. M., Hedhammar, Å. & Lindblad-Toh, K. (2013). The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature*, 495 (7441): 360-364.

Badrie, N. & Mellowes, W. (1991). Effect of extrusion variables on cassava extrudates. *Journal of Food Science*, 56 (5): 1334-1337.

BAŞER, Ö. & Yalcin, S. (2017). Determination of some quality characteristics in pet foods. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 64 (1): 21-24.

Behera, S., Singh, R., Arora, R., Sharma, N. K., Shukla, M. & Kumar, S. (2015). Scope of algae as third generation biofuels. *Frontiers in bioengineering and biotechnology*, 2: 90.

Bhagat, R., Sheikh, A. A., Wazir, V., Mishra, A. & Maibam, U. (2017). Food allergy in canines: A review. *Journal of Entomology and Zoology Studies*, 5 (6): 1522-1525.

Bosch, G., Zhang, S., Oonincx, D. G. & Hendriks, W. H. (2014). Protein quality of insects as potential ingredients for dog and cat foods. *Journal of nutritional science*, 3.

Bosch, G. & Swanson, K. (2020). Effect of using insects as feed on animals: pet dogs and cats. *Journal of Insects as Food and Feed*: 1-12.

BusinessWire. (2021). *Pet Food Global Market Report 2021: COVID-19 Impact and Recovery to 2030*.

Case, L. P., Daristotle, L., Hayek, M. G. & Raasch, M. F. (2010). *Canine and Feline Nutrition-E-Book: A Resource for Companion Animal Professionals*: Elsevier Health Sciences.

Clutton-Brock, J. (1990). A Natural History of Domesticated Mammals. *Praehistorische Zeitschrift*, 65 (1): 73-76.

Crane, S., Cowell, C., Stout, N., Moser, E., Millican, J., Romano, P. & Crane, S. (2010). Commercial pet foods. *Small animal clinical nutrition*: 157-190.

Darwin, C. (2010). *The variation of animals and plants under domestication*, vol. 2: Cambridge University Press.

Day, L., Augustin, M., Batey, I. & Wrigley, C. (2006). Wheat-gluten uses and industry needs. *Trends in food science & technology*, 17 (2): 82-90.

De Silva, S., Francis, D. S. & Tacon, A. G. (2011). Fish oil in aquaculture: in retrospect. *Fish oil replacement and alternative lipid sources in aquaculture feeds*: 1-20.

De Silva, S. S. & Turchini, G. M. (2008). Towards understanding the impacts of the pet food industry on world fish and seafood supplies. *Journal of agricultural and environmental ethics*, 21 (5): 459-467.

Draganovic, V., van der Goot, A. J., Boom, R. & Jonkers, J. (2011). Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. *Animal Feed Science and Technology*, 165 (3-4): 238-250.

Draganovic, V., Van der Goot, A., Boom, R. & Jonkers, J. (2013). Wheat gluten in extruded fish feed: effects on morphology and on physical and functional properties. *Aquaculture nutrition*, 19 (6): 845-859.

Driscoll, C. A., Macdonald, D. W. & O'Brien, S. J. (2009). From wild animals to domestic pets, an evolutionary view of domestication. *Proceedings of the National Academy of Sciences*, 106 (Supplement 1): 9971-9978.

Ekmay, R., Gatrell, S., Lum, K., Kim, J. & Lei, X. G. (2014). Nutritional and metabolic impacts of a defatted green marine microalgal (*Desmodesmus* sp.) biomass in diets for weanling pigs and broiler chickens. *Journal of agricultural and food chemistry*, 62 (40): 9783-9791.

EuromonitorInternational. (2018). *Innovation in Pet Food: Premiumisation and Segmentation*.

Fan, J., Mitchell, J. R. & Blanshard, J. M. (1996). The effect of sugars on the extrusion of maize grits: I. The role of the glass transition in determining product density and shape. *International journal of food science & technology*, 31 (1): 55-65.

Frame, N. D. & Harper, J. M. (1994). *The technology of extrusion cooking*: Springer.

Gatlin III, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogdahl, Å. & Nelson, R. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture research*, 38 (6): 551-579.

Gatrell, S., Lum, K., Kim, J. & Lei, X. (2014). Potential of defatted microalgae from the biofuel industry as an ingredient to replace corn and soybean meal in swine and poultry diets. *Journal of Animal Science*, 92 (4): 1306-1314.

Ghasemi, A. & Zahediasl, S. (2012). Normality tests for statistical analysis: a guide for non-statisticians. *International journal of endocrinology and metabolism*, 10 (2): 486.

Ghorpade, V., Bhatnagar, S. & Hanna, M. (1997). Structural characteristics of corn starches extruded with soy protein isolate or wheat gluten. *Plant foods for human nutrition*, 51 (2): 109-124.

Gong, Y., Guterres, H., Huntley, M., Sørensen, M. & Kiron, V. (2018). Digestibility of the defatted microalgae Nannochloropsis sp. and *Desmodesmus* sp. when fed to Atlantic salmon, Salmo salar. *Aquaculture Nutrition*, 24 (1): 56-64.

Gong, Y., Bandara, T., Huntley, M., Johnson, Z. I., Dias, J., Dahle, D., Sørensen, M. & Kiron, V. (2019). Microalgae Scenedesmus sp. as a potential ingredient in low fishmeal diets for Atlantic salmon (Salmo salar L.). *Aquaculture*, 501: 455-464.

Hemmer, H. (1990). *Domestication: the decline of environmental appreciation*: Cambridge University Press.

Jin, Z., Hsieh, F. & Huff, H. (1995). Effects of soy fiber, salt, sugar and screw speed on physical properties and microstructure of corn meal extrudate. *Journal of Cereal Science*, 22 (2): 185-194.

Kienzle, E. (1994a). Blood sugar levels and renal sugar excretion after the intake of high carbohydrate diets in cats. *The Journal of nutrition*, 124 (suppl_12): 2563S-2567S.

Kienzle, E. (1994b). Effect of carbohydrates on digestion in the cat. *The Journal of nutrition*, 124 (suppl_12): 2568S-2571S.

Kwak, M.-K. & Cha, S.-S. (2021). A Study on the Selection Attributes Affecting Pet Food Purchase: After COVID-19 Pandemic. *International Journal of Food Properties*, 24 (1): 291-303.

Manor, M. L., Kim, J., Derksen, T. J., Schwartz, R. L., Roneker, C. A., Bhatnagar, R. S. & Lei, X. G. (2017). Defatted microalgae serve as a dual dietary source of highly bioavailable iron and protein in an anemic pig model. *Algal research*, 26: 409-414.

Matassa, S., Boon, N., Pikaar, I. & Verstraete, W. (2016). Microbial protein: future sustainable food supply route with low environmental footprint. *Microbial biotechnology*, 9 (5): 568-575.

Murphy-Bokern, D. (2010). Understanding the carbon footprint of our food. *Complete Nutrition*, 10: 61-3.

O'Rourke, D. & Ringer, A. (2016). The impact of sustainability information on consumer decision making. *Journal of Industrial Ecology*, 20 (4): 882-892.

Olguín, E. J. (2012). Dual purpose microalgae–bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a Biorefinery. *Biotechnology advances*, 30 (5): 1031-1046.

Peters, J., von den Dreisch, A. & Helmer, D. (2005). *The upper Euphrates-Tigris basin: cradle of agro-pastoralism?*: na.

Ramaraj, S., Hemaiswarya, S., Raja, R., Ganesan, V., Anbazhagan, C., Carvalho, I. S. & Juntawong, N. (2015). Microalgae as an attractive source for biofuel production. In *Environmental Sustainability*, pp. 129-157: Springer.

Refstie, S. & Tiekstra, H. A. (2003). Potato protein concentrate with low content of solanidine glycoalkaloids in diets for Atlantic salmon (Salmo salar). *Aquaculture*, 216 (1-4): 283-298.

Renewables 2020. (2020). IEA, Paris.

Riaz, M. N. & Rokey, G. J. (2011). *Extrusion problems solved: Food, pet food and feed*: Elsevier.

Rokey, G. J., Plattner, B. & Souza, E. M. d. (2010). Feed extrusion process description. *Revista Brasileira de Zootecnia*, 39: 510-518.

Romsos, D. R., Palmer, H. J., Muiruri, K. L. & Bennink, M. R. (1981). Influence of a low carbohydrate diet on performance of pregnant and lactating dogs. *The Journal of nutrition*, 111 (4): 678-689.

Rothgerber, H. (2013). A meaty matter. Pet diet and the vegetarian's dilemma. *Appetite*, 68: 76-82.

Rothgerber, H. (2014). Carnivorous cats, vegetarian dogs, and the resolution of the vegetarian's dilemma. *Anthrozoös*, 27 (4): 485-498.

Royston, P. (1995). Remark AS R94: A remark on algorithm AS 181: The W-test for normality. *Journal of the Royal Statistical Society. Series C (Applied Statistics)*, 44 (4): 547-551.

Rozin, P., Markwith, M. & Stoess, C. (1997). Moralization and becoming a vegetarian: The transformation of preferences into values and the recruitment of disgust. *Psychological science*, 8 (2): 67-73.

Ryu, G.-H. (2020). Extrusion cooking of high-moisture meat analogues. *Extrusion Cooking: Cereal Grains Processing*: 205.

Serpell, J. A. (2000). Domestication and history of the cat. *The domestic cat: The biology of its behaviour*, 2: 180-192.

Spears, J. K. & Fahey Jr, G. C. (2004). Resistant starch as related to companion animal nutrition. *Journal of AOAC International*, 87 (3): 787-791.

Sunquist, M. & Sunquist, F. (2017). *Wild cats of the world*: University of chicago press.

Swanson, K. S., Carter, R. A., Yount, T. P., Aretz, J. & Buff, P. R. (2013). Nutritional sustainability of pet foods. *Advances in Nutrition*, 4 (2): 141-150.

Sørensen, M., Stjepanovic, N., Romarheim, O., Krekling, T. & Storebakken, T. (2009). Soybean meal improves the physical quality of extruded fish feed. *Animal Feed Science and Technology*, 149 (1-2): 149-161.

Sørensen, M., Berge, G. M., Thomassen, M. S., Ruyter, B., Hatlen, B., Ytrestøyl, T., Aas, T. S. & Åsgård, T. E. (2011). Today's and tomorrow's feed ingredients in Norwegian aquaculture. *Nofima rapportserie*.

Thomas, M. & Van der Poel, A. (1996). Physical quality of pelleted animal feed 1. Criteria for pellet quality. *Animal feed science and technology*, 61 (1-4): 89-112.

Tyler, J. S., J. (2020, 03/02/2020). Top 5 trends seen at Global Pet Expo 2020. *Pet Food Processing*. Available at: https://www.petfoodprocessing.net/articles/13659-top-5-trends-seen-at-global-pet-expo-2020.

Uribe-Wandurraga, Z. N., Igual, M., García-Segovia, P. & Martínez-Monzó, J. (2020). Influence of microalgae addition in formulation on colour, texture, and extrusion parameters of corn snacks. *Food Science and Technology International*, 26 (8): 685-695.

Valente, L. M. P., Custódio, M., Batista, S., Fernandes, H. & Kiron, V. (2019). Defatted microalgae (Nannochloropsis sp.) from biorefinery as a potential feed protein source to replace fishmeal in European sea bass diets. *Fish physiology and biochemistry*, 45 (3): 1067-1081.

Van der Spiegel, M., Noordam, M. & Van der Fels-Klerx, H. (2013). Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. *Comprehensive reviews in food science and food safety*, 12 (6): 662-678.

Verlinden, A., Hesta, M., Millet, S. & Janssens, G. (2006). Food allergy in dogs and cats: a review. *Critical reviews in food science and nutrition*, 46 (3): 259-273.

Vilà, C., Savolainen, P., Maldonado, J. E., Amorim, I. R., Rice, J. E., Honeycutt, R. L., Crandall, K. A., Lundeberg, J. & Wayne, R. K. (1997). Multiple and ancient origins of the domestic dog. *Science*, 276 (5319): 1687-1689.

White, S. D. (1988). Food hypersensitivity. *Veterinary Clinics of North America: Small Animal Practice*, 18 (5): 1043-1048.

Wojnowska, I., Poznanski, S. & Bednarski, W. (1982). Processing of potato protein concentrates and their properties. *Journal of Food Science*, 47 (1): 167-172.

Xie, S. & Jokumsen, A. (1997). Incorporation of potato protein concentrate in diets for rainbow trout: effect on feed intake, growth and feed utilization. *Aquaculture Nutrition*, 3 (4): 223-226.

Xie, S. & Jokumsen, A. (1997). Replacement of fish meal by potato protein concentrate in diets for rainbow trout, Oncorhynchus mykiss (Walbaum): growth, feed utilization and body composition. *Aquaculture Nutrition*, 3 (1): 65-69.

Zafalon, R. V. A., Risolia, L. W., Vendramini, T. H. A., Ayres Rodrigues, R. B., Pedrinelli, V., Teixeira, F. A., Rentas, M. F., Perini, M. P., Alvarenga, I. C. & Brunetto, M. A. (2020). Nutritional inadequacies in commercial vegan foods for dogs and cats. *PloS one*, 15 (1): e0227046.

Zeuner, F. E. (1963). A history of domesticated animals. *A history of domesticated animals.*

Zoran, D. L. (2002). The carnivore connection to nutrition in cats. *Journal of the American Veterinary Medical Association*, 221 (11): 1559-1567.

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