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Effect of using insects as a dogfood ingredient: A perspective on technology and nutrition on focus to *Lumbriculus variegatus, Hermetia illucens* and *Tenebrio molitor*

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Effect of using insects as a dogfood ingredient: A perspective on technology and nutrition on focus to *Lumbriculus variegatus*, *Hermetia illucens* and *Tenebrio molitor*- A literature review

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Abstract

The use of insect as food and feed has been in existence for a very long time as it was used as an alternative protein source for food and feed production. The current food crises has caused researchers to find ways of maximizing the production of insect to supplement some of the non conventional protein source. Insect also have high feed conversion ratio and requires less technology to produce. The blackworm (*Lumbriculus variegatus*) is considered a good protein source for nutrient trial application as it possesses a good nutritional profile. On the average, the black worm contains about 47.8-68% protein and 7-25% lipids which is considered good for the production of dog food. the black worm also possesses other nutrients which is beneficial for growth and development of the dogs. Also, the black soldier fly has been one of the most used insect for feed trials as well as used as food by humans. It contains about 40.1-63% protein, and 12.8-49% lipids as well as other nutrials which helps improve the wellbeing of animals. On the other hand, the mealworm (*Tenebrio molitor*) possesses averagely 47-55.30% protein and 22.97-37.70% lipids as well as other nutritional components useful for dog growth and deelopment. Before insect is ready to be used as feed, they need to undergo processing in other to make it easier to produce the entire dog diet and this is due to its high fat content. The insect meal goes through rheological changes during production of the dog diets and processing parameters needed to be adjusted continuously to improve quality of the extruded diets. Using insect meal as a feed ingredient also has its challenges such as consumer acceptance, price, environmental conditions, safety and allergens

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1. Introduction

The current food crisis has steered the direction of scientists towards the use of insects as food and feed. Consumption of insects as food and feed has been in existence for centuries, making it one of the oldest sources of proteins. Thus, it is estimated that by 2050, demand for protein from animal sources will increase as a result of the ever-increasing of human population and an increase in the standard of living of people (Bosch *et al.*, 2014). Such high demand will increase the competition between humans and animals for protein in their diets. Hence, to ensure food security in protein, a more sustainable protein source needs to be developed. Large-scale production of most insects is potentially possible as they can be grown on organic waste streams such as decomposing waste and cow dug (Van Huis, 2020). Furthermore, about 6% of pets diet worldwide is known to contain insect as their primary or partial protein source (Plantinga *et al.*, 2011). Entomophagy, which is also known as the consumption of insects, has been practiced by humans on every inhabited continent throughout history and today (Wang & Shelomi, 2017). Insects play a larger role in the cuisines of some contemporary cultures (Hanboonsong, 2010), while they are taboo or at the very least unappealing in other cultures (Yen, 2009). However, discussing the role insects should play in modern, urban, or western food culture has increased over the last century, perhaps beginning with (Holt's 1885) essay, "Why Not Eat Insects?". Without a long history of entomophagy, consumers are becoming more aware that insects are edible and offer advantages over other protein sources. Thus, and are expressing an increased interest in trying edible insect dishes (Wang & Shelomi, 2017), whether out of curiosity (DeFoliart, 1988), environmental concerns (Tucker 2014), or other reasons (Verbeke, 2015). The popularity of entomophagy has grown to the point that insect supply cannot keep up with demand (Gracer, 2010; Tanter, 2013), and several edible species are already threatened by overexploitation (Johnson, 2010). International conferences and cookbooks have suggested using insects as alternative protein sources (Wang & Shelomi, 2017). The potential of some edible insects as a solution to current or looming food crises (Gahukar, 2011; Martin, 2014; Nadeau *et al.*, 2015), in particular fears of global food insecurity due to climate change and rising populations (Shelomi, 2016; Van Huis, 2013), has gotten a lot of attention. Insects are high in proteins, healthy fats, and trace elements, in addition to being tasty when prepared properly. Their main benefit over other protein sources, which explains why they're frequently hailed as food-security saviours, is their decreased environmental effect (Wang &

Shelomi, 2017). The feed-to-protein conversion ratio is lower than cattle, pigs, and even poultry, according to some sources (Van Huis, 2013), and create fewer greenhouse gases and ammonia emissions than any other livestock (Van Huis, 2013; Costa-Neto, 2014; Oonincx *et al*., 2015). According to Makkar *et al.* (2014), insects have a high feed conversion ratio and are easy to produce and grow due to their cold-blooded nature. Insect rearing may be done in underdeveloped nations with low-technology and low-capital investment while producing reliable, safe, and high-quality goods using high-technology and automated procedures (Van Huis *et al.*, 2013). Collavo *et al*. (2005) also added that, on an average of 2 kg of bio-waste, one kg of insect meal, making it highly productive. Insects can indirectly reduce the environmental impact of vertebrate meats by serving as a source of food (Singh-Ackbarali & Maharaj, 2017; Makkar *et al*., 2014). However, one known issue with industrializing edible insects today is the scarcity of available insects (Wang & Shelomi, 2017). While thousands of species are consumed globally (Nadeau *et al.*, 2015), all but a dozen or so are caught in the wild by more traditional societies and cannot be farmed at this time, posing problems for regular supply and conservation (Ferreira, 1995; DeFoliart, 1995). House crickets and mealworms, for example, are not always the most sustainable or have the most desirable properties like taste, texture and organoleptic properties (Wang & Shelomi, 2017). Several authors have stated that, when done incorrectly, entomophagy can be harmful to the environment rather than beneficial (Gahukar, 2011; Ferreira, 1995). Edible insects that are difficult to raised or harvest and thus in short supply would be more expensive, making it less desirable among most consumers (Wang & Shelomi, 2017). Raising insects on otherwise inedible organic wastes would significantly reduce their environmental impact and increase their utility, particularly for developing world-consumers (Wang & Shelomi, 2017). The recycling of waste is an added benefit as organic waste management such as leachates, manure, and food waste is both expensive and a growing environmental concern (Popa & Green, 2012). Raising edible insects on waste would solve two problems at once (Salomone, 2017), but popular species such as cricket and mealworm are difficult to rear on most waste, particularly animal products (Wang & Shelomi, 2017). As a result, it has become important to identify and refocus attention on species with higher cultivation characteristics than extant edible insect species but can still be utilized as feed (Wang & Shelomi, 2017).

Research conducted by Veldkamp *et al.* (2012) concluded that insects could be produced on a large scale as an alternative sustainable protein source for ruminants and monogastric when reared on organic waste streams. Using insects as animal feed is currently being accepted in

Europe as the need to find alternative protein sources to soybean and fishmeal rises (Verbeke, 2015). Insect meal is considered as an alternative protein source in the diet of pets (Wang & Shelomi, 2017). Despite the imposition of legislative barriers (Regulation (EC) No. 1069/2009) with regards to the inclusion of insect meal in livestock diets, the European Union accepts insect meal as a processed animal protein (Kierończyk *et al.,* 2018). Some commercial feed producers include insects in their products, mainly in the dried form, to make the feed more attractive to pets (Wang & Shelomi, 2017). The innovative application of insects as a protein ingredient is gradually becoming an exciting topic of discussion regarding pet nutrition, usually when used in hypoallergenic feeds where chicken by-products and soybean meal is excluded (Kierończyk *et al.*, 2018). However, antimicrobial peptide and chitin in insects make it an ideal functional feed component (Jozefiak *et al.,* 2016; Jozefiak and Engberg, 2017). In an experiment conducted by Kierończyk *et al.* (2018), insects play an essential role as an alternative aroma additive to commercial feed aroma additives.

However, the information on incorporating *Lumbriculus variegatus, Hermetia illucens*, and *Tenebrio molitor* in dog food are limited. This study reviews the effect of using *Lumbriculus variegatus, Hermetia illucens*, and *Tenebrio molitor* in dog foods focusing on technology and nutrition.

2. Nutritional composition of *Lumbriculus variegatus, Hermetia illucens* and *Tenebrio molitor*

2.1.1. Black worm- *Lumbriculus variegatus* (*L. variegatus*)

Lumbriculus variegatus is a widespread oligochaete that lives in rivers, wetlands, lakes, and marshes (Gustafsson *et al.,* 2009). The genital apparatus of *L. variegatus* has been described in the past, but recent reports of sexually mature individuals are scarce, at least in Europe (Gustafsson *et al.,* 2009). There are several extremely desirable characteristics of *Lumbriculus variegatus* (Mount *et al.,* 2006). Methods of culture for *L. Variegatus* is well established (U.S. Environmental Protection Agency, 2000; American Society for Testing and Materials, 2004) and may readily be grown with a minimum of facility and/or care at extremely high densities (Mount *et al.,* 2006). Doubling time can be quite quick in the presence of enough feeding (10-14 days) (Mount *et al.,* 2006). Chemical dosage can be achieved through exposure to water, to sediment, or both, and by an apparently poor xenobiotic metabolism in *L. Variegatus* helps keep their tissue dosed chemical (Ankley & Collyard, 1995; Guerrero *et al.,* 2002; Mount *et al.,* 2006). The quality of *L. variegatus* is good for use in nutrient exposure trial applications (Abel *et al.,* 2017). They are easily cultivated, are quickly accepted by fish, encourage good growth and survival in investigated fish species, are easily loaded with toxicants, remain healthy and appetizing long after the fish have been introduced and are conducive to precise food intake measurement (Mount *et al.,* 2006). It is therefore anticipate that other researchers will find them very beneficial in investigations of dietary chemicals consumption and toxicity. However, research works on its use as a dog food ingredient is limited. Table 1 indicates the nutritional composition of *Lumbriculus variegatus*

Table 1. Nutritional composition of *Lumbriculus variegatus* (freeze-dried type)

Compound	Amount	Unit	Source
Lipids	7.22	%	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
	7-25		Elissen *et al*., (2015)
	20.1		Macke, 2013
	11-12		Elissen *et al*., (2010)
Ashes	5.91	%	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
	5-11		Elissen *et al*., (2015)
	9-11		Elissen *et al*., (2010)
Protein	59.3	%	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
	62-68		Elissen *et al*., (2015)
	47.8		Macke, 2013
	62-66		Elissen *et al*., (2010)
Moisture	7.34	%	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
Carbohydrates (calculated)	20.2	%	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
EPA	4.53	% of total fatty acids	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
DPA	1.79	% of total fatty acids	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
Monounsaturated fatty acids	20.9	% of total fatty acids	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
Polyunsaturated fatty acids	28	% of total fatty	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
	17-45		Elissen et al., (2015)

		acids	
Omega 3 fatty acids	8.13	% of total fatty acids	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
	8-21		Elissen et al., (2015)
Omega 6 fatty acids	18.3	% of total fatty acids	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
	15-23		Elissen et al., (2015)
Docosahexaenoic acid	1-5	% of total fatty acids	Elissen et al., (2015)
Eicosapentaenoic acid	4-12	% of total fatty acids	Elissen et al., (2015)
Omega 3: omega 6 ratio	0.4-0.9		Elissen et al., (2015)
Energetic value	1630	kJ/100 g	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
Energetic value	387	Kcal/100 g	https://www.blackworms.com.au/SearchResults.asp?Cat=1821
	4-7		Macke, 2013

2.1.2. Black soldier fly larvae- *Hermetia illucens*

Hermetia illucens, which is also known as the black soldier moth, is a true fly (Diptera) belonging to the *Stratiomyidae* tribe. Although it is originally a native to the Americas, it now thrives in tropical and temperate climates worldwide (Sheppard *et al.*, 1994; Čičková *et al*., 2015). Its lack of cold hardiness prevents it from spreading to non-native areas like Northern Europe (Spranghers *et al.*, 2017). Adults drink only water, do not approach humans, do not bite or sting, and do not transmit or vector any pathogens (Čičková *et al*., 2015; Sheppard *et al.,* 1994). Black soldier fly larvae (BSFL) have been employed in small-scale waste management utilizing substrates such as manure (Sheppard, 1983; Yu, 2009), rice straw (Zheng. 2012), food waste (Green & Popa, 2012), distillers' grains (Webster *et al.,* 2016), fecal sludge (Lalander, 2013; Banks *et al.,* 2014), animal and kitchen waste (Nguyen *et al.,* 2015). They may be the most diverse in terms of substrates they can digest and their efficiency among the flies (Kim *et al.*, 2011). BSFLs are edible and have been explored in this regard. Compared to crickets and mealworms, their feed conversion ratios are more significant. Their survival rate and nitrogen and phosphorus contents do not fluctuate like other insects (Oonincx *et al.,* 2015). They aren't considered harmless (Blum, 1994). BSFL accrues lipids from their food for the non-feeding adult to consume as energy, to the point where they can be turned into biodiesel (Li *et al.,* 2011; Wang *et al.,* 2017; Nguyen *et al.,* 2017; Mohn-Noor *et al.,* 2017). What they don't eat can be utilized as fertilizer, together with their nitrogen-rich frass (Green & Popa, 2012). Their larval growth phase is more prolonged than flies like house and carrion flies (five days), implying that a single larva would use more substrate and generate more giant pupae (Čičková *et al.,* 2015). All of these advantages make BSFL a viable livestock feed or human food source, as well as a valuable tool for waste valorization (Wang & Shelomi, 2017). Table 2 and 3 indicates the nutritional composition and amino acid profile of *Hermetia illucens* respectivefully.

Table 2. Nutritional composition of *Hermetia illucens* (Dried type)

Compounds	Amount	Unit	Source
Dry matter	100	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
Moisture	590-619	g/kg	Spranghers *et al.,* (2017)
Crude protein	41.1	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
	399-431	g/kg	Spranghers *et al.,* (2017)
	17.5-63	%	Shelomi, 2020
	40.1- 62.7		Barragan-Fonseca *et al.,* (2017)
Crude fibre	9.4	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
Crude fat	35.5	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
	12.8-49	%	Shelomi, 2020
	27-197	g/kg	Spranghers *et al.,* (2017)
	30	%	St-Hilaire *et al.,* (2007)
	6.63-34.8		Barragan-Fonseca *et al.,* (2017)
Ash	11.7	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
	27-197	g/kg	Spranghers *et al.,* (2017)

Insoluble ash	0.6	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
NDF	17.4	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
ADF	9.2	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
Saturated fatty acid	28.1-38.43		Shelomi, 2020
Palmitic acid	22	%	Shelomi, 2020
Omega-3 fatty acid	3	%	St-Hilaire *et al.*, (2007)
Lignin	2.7	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
Chitin	377-407	g/kg	Spranghers *et al.*, (2017)
	21	g/kg	Finke, 2013
Water-insoluble cell walls	16.6	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
Starch	0.9	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
Starch, enzymatic method	0	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
Total sugars	0	%	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
Gross energy (kcal)	6170	Kcal/kg	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0
	199.4	Kcal/100g	Finke, 2013
Gross energy (MJ)	25.8	MJ/kg	https://feedtables.com/content/black-soldier-fly-larvae-fat-20-dried-0

Table 3. Amino acid profile of *Hermetia illucens*

Amino acid	**Amount**	**sources**
Arginine	[a]16.4; [a]93.31; [b]3.9	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Histidine	[a]4.7; [a]14.76; [b]2.2	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Isoleucine	[a]22.4; [a]12.22; [b]3.3	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Leucine	[a]33.0; [a]37.33; [b]5.2	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Lysine	[a]19.6; [a]28.63; [b]3.8	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
cystine	[a]0.9; [a]89.17; [b]0.1	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Methionine	[a]6.2; [a]26.46; [b]2.1	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Phenylalanine	[a]16.9; [a]16.29; [b]3.0	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
tyrosine	[a]27.0; [a]26.97; [b]4.8	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Threonine	[a]19.3; [a]22.36; [b]3.1	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Tryptophan	[a]0.2; [a]0.49	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016
Valine	[a]35.8; [a]21.93; [b]4.9	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Alanine	[a]46.4; [a]52.45; [b]6.2	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Aspartic acid	[a]44.9; [a]48.08; [b]6.7	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Glycine	[a]44.3; [a]10.9; [b]4.2	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Glutamic acid	[a]45.2; [a]65.81;	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et*

	[b]8.8	*al.*, 2020
Proline	[a]29.1; [a]36.65; [b]5.5	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
Serine	[a]21.3; [a]32.88; [b]3.7	Cullere *et al.*, 2016; Al-Qazzaz *et al.*, 2016; Stejskal *et al.*, 2020
[a] g/kg [b] %		

2.1.3. Mealworm- *Tenebrio molitor (T. molitor)*

T. molitor is a worldwide parasite of flour, grain, and other food crops (Ramos-Elorduy *et al*., 2002). It's a darkling beetle species with four stages of development: egg, larva, pupa, and adult. *T. molitor* females lay about 500 eggs, which hatch in 3–9 days and become larvae (Lee *et al.,* 2019; Siemianowska *et al.,* 2013). The ideal incubation temperature is 25°C to 27°C, where embryonic development takes 4–6 days (Siemianowska *et al.,* 2013). The hatching process can be accelerated by slightly raising the temperature. This species has a very long larval growth, which takes about half a year at the optimum temperature and moisture level. Larvae eat deep inside the materials, preventing sunshine. Larvae emerge onto the surface of food items and transform into pupae just before the end of this stage. The pupal period lasts around 5 to 6 days (Siemianowska *et al.,* 2013). Mealworms are an intriguing source of protein in feeding fish, poultry and pig (Veldkamp *et al.,* 2012). *T. molitor* is a pest of stored flour, grain and feed and is high in protein (46% to 60%) and simple to reproduce in larval and pupal stages (Siemianowska *et al.,* 2013). The grade of protein is comparable for soyabean meal, but the level of methionine in poultry feed is restricted (Ramos-Elorduy *et al.,* 2002). *T. molitor* contains chitin, an arthropod exoskeleton polysaccharide (Hensel *et al*., 2015) which cannot be digested by monogastric animals (Sánchez-Madrigal *et al.,* 2014). The usage of antibiotics can be reduced through the feed-in of black soldiers flying larvae, mealworm and field cricket to chicken, but this can positively impact the health of poultry (Siemianowska *et al.,* 2013). van Huis (2013) has noted that antibiotics may be decreased because diets that include around 3% of chitin increased intestinal population and decreased intestinal populations *of Lactobacillus spp*. and *Escherichia coli*. Siemianowska *et al.* (2013), found that *T.molitor* does not influence palatability and bird performance up to 10% in a broiler diet. Few researches on insect food incorporation in dog diets are nonetheless known thus, more research need to be conducted in such area. Table 4 and 5 shows the nutritional compositions and amino acid profile of *Tenebrio molitor* respectivefully.

Table 4. Nutrient composition of *Tenebrio molitor*

Component	**Unit**	**Amount**	**Source**
Crude protein	%	55.27	De Marco *et al.*, (2015)
		55.30	Bovera *et al.*, (2016)
		53.83	Sedgh-Gooya., (2020)
		47.70	Ramos-Elorduy *et al.*, (2002)
		50.79	Yoo *et al.*, (2019)
		47.00	Benzertiha *et al.*, (2020)
Crude fat	%	29.54	De Marco *et al.*, (2015)
		22.97	Bovera *et al.*, (2016)
		28.03	Sedgh-Gooya., (2020)
		37.70	Ramos-Elorduy *et al.*, (2002)
		36.77	Yoo *et al.*, (2019)
		29.60	Benzertiha *et al.*, (2020)
Ash	%	4.99	Bovera *et al.*, (2016)
		6.99	Sedgh-Gooya., (2020)
		6.70	Yoo *et al.*, (2019)
		2.56	Benzertiha *et al.*, (2020)
Crude fibre	%	7.53	Sedgh-Gooya., (2020)
		5.00	Ramos-Elorduy *et al.*, (2002)
		6.48	Yoo et al., (2019)
		5.60	Benzertiha *et al.*, (2020)
Acid detergent fibre	%	7.66	Bovera *et al.*, (2016)
Chitin	%	5.60	Sedgh-Gooya., (2020)
		8.91	Benzertiha *et al.*, (2020)
Gross energy	kcal	214	Nowak, 2016
		247	Payne *et al.*, (2016)

Table 5. Amino acid profile of *Tenebrio molitor*

Amino acid	**Amount (%)**						
Arginine	2.23	2.43	2.23	4.42	2.21	1.89	2.51
Histidine	2.80	1.53	1.38	2.77	1.65	0.84	1.41
Isoleucine	1.98	3.56	1.83	6.48	4.51	1.31	2.08
Leucine	3.37	3.41	3.13	6.21	5.32	2.21	3.78
Lysine	2.01	2.91	2.50	5.31	4.51	1.58	2.65
cystine	3.16	0.52		0.93		1.19	0.58
Methionine		0.67	0.52	1.22	1.34	0.60	0.73
Phenylalanine	1.76	1.76	1.55	3.20	1.54	1.31	1.93
tyrosine	3.45	3.46		6.32	2.32	2.15	3.14
Threonine	1.83	1.81	1.70	3.31	1.64	1.27	2.51
Tryptophan				0.02		0.30	0.47
Valine	2.94	2.44	2.57	4.46	4.42	1.89	3.33
Alanine	3.96	3.69		6.70	4.34	2.48	4.17
Aspartic acid	2.76	3.59		6.52	3.23	1.54	3.82
Glycine	2.61	2.41		4.38	2.65	1.71	2.65
Glutamic acid	5.78	5.68		10.32	4.75	3.92	6.03
Proline	1.66	3.02		10.32	4.75	3.92	3.13
Serine	2.20	2.09	2.23	3.82	3.45	1.36	2.28
Sources	Ghosh *et al.,* (2017)	Ravzanaadii *et al.,* (2012)	Heidari-Parsa, 2018	Ao *et al.,* (2020)	Hussain *et al.,* (2017)	Wu *et al.,* (2020)	Benzertiha *et al.,* (2020)

2.2. Nutrient requirements for dogs

Dogs belong to the order Carnivora even though it has evolved to consume omnivores' diets (Rooney & Stafford, 2018). According to Bosch *et al.* (2015), the dog diet was based on anecdotal experience from domestication until the beginning of the twentieth century. Over the past years, a significant increase in research into dog nutrition, especially nutritional requirements, has been recorded (McGreevy *et al.*, 2005). The keystone of rationing is determining maintenance energy needs as well as assessing the energy quality of food (Guo *et al.*, 2021). When looking at the extensive literature on dog nutrition, it is worth noting that, in contrast to those on mineral, vitamin, and protein needs, comparatively few works have been published on this crucial subject (NRC, 1995). When implemented to each canine breed, nutritional criteria suggested by McCay (1943) are not accurate. Dogs require glucose precursors, fatty acids, specific amino acids, and essential dietary components for healthy living (Zoran, 2002). Although dogs tend to synthesize their own vitamin C, their diets are supplemented with minerals and vitamins (Case *et al.,* 2010). According to Brown (1989), dietary protein can be grouped as proteins that provide amino acids from which the animal can make proteins and as a source of flavour. To determine the appropriate nutrients requirement for dogs, their health condition, lifestyle, activity level, and dog's age must be considered (Rooney & Stafford, 2018; Fahey *et al.*, 2008; Hill *et al.,* 2009). Furthermore, dogs have a wide variation concerning their body weight, making their energy requirement a bit difficult to estimate even though this variation also presents an opportunity to determine the energy requirement of dogs with a wide range of body types and shapes (Finke, 1991).

According to Hill (1998), fat oxidation supplies most energy in dogs, as it does in other mammals, at low rates of energy expenditure (60 % at 40 % of maximal oxygen uptake). Glucose oxidation increases as exercise intensity rises, while fat oxidation remains stable, making glucose oxidation is the primary source of energy at elevated rates of energy expenditure (80 p% at 85 percent) (Weibel *et al.,* 1996). On the other hand, dogs burn half as much fat as humans and goats do at rest and during exercise (McLelland *et al*., 1994; Meyer and Doty 1998).

For race dogs, dietary fiber can have certain health benefits. Water and electrolyte absorption is aided by volatile fatty acids formed by bacterial fermentation of soluble fiber in the canine colon (Herschel *et al.,* 1981). Rapid oligosaccharide fermentation can lower colonic pH and inhibit clostridial development, aided by meat consumption (Amtsberg *et al*. 1989). In extruded dog diets, carbohydrate digestibility varies, although not all starch is digested in the canine small intestine (Schunemann *et al.,* 1989). The colon of dogs fed beef and corn starch diets, and no fiber has a high concentration of volatile fatty acids (Hill 1998). Increased dietary fiber decreases nutritional supply while increasing fecal weight. As a result, the effects of adding more fiber to canine athletes remain unknown (Hill, 1998)

Most commercial pet foods have enough vitamins and minerals for sedentary dogs, although the vitamin and mineral balance will need to be adjusted for exercise dogs (Wakshlag & Shmalberg, 2014). Endurance running dogs, who eat a lot of food, can demand fewer vitamins and minerals per joule than greyhounds, which consume around the same amount of food, vitamins, and minerals as sedentary dogs (Hill, 2010).

Protein is, however, used as a binding agent in the dry pet food industry to improve pellet structure (Manbeck *et al.*, 2017). Also, in canned foods, the product's juicy appearance is due to the moisture-binding property of the proteins present in them (Brown, 1989).

Table 6 indicates some standard ingredients and their nutritional composition used in the production of dog food.

Table 6. Standard Ingredients and amount required for the production of dog food

Ingredients	**g/kg**		**Chemical composition**	**g/kg**
Wheat	273.1		Dry matter	914.6
Corn	253.0		Starch	326.8
Chicken meal	120.0		Crude protein	253.2
Meat meal	65.0		Crude fat	111.3
Liquid fat (bovine, pig)	64.0		Crude ash	65.5
Maize gluten feed	50.0		Neutral detergent fibre	85.9
Chicory	50.0		Reducing sugars	32.6
Poultry meal, hydrolysed	30.0			
Bloodmeal	25.0			
Maltodextrin	25.0			
Vitamin-mineral premix[a]	25.0			
Yeast[b]	5.0			
Salmon oil	5.0			
Pellet binder[c]	5.0			
Organic acids[d]	2.0			
Sunflower oil	2.0			
Emulgator (liquid)[e]	0.5			
Fructo-oligosaccharides[f]	0.4			

[a] Premix, provided per kg of diet: Vitamin A (retinol), 15,000 IU; Vitamin D3 (cholecalciferol), 1500 IU; Vitamin E (all-rac-alpha-tocoferyl acetate), 100 IU; Vitamin B1 (thiamine mononitrate), 12 mg; vitamin B2 (riboflavin), 12mg; vitamin B6 (pyridoxine hydrochloride), 10 mg; vitamin B12 (cyanocobalamin), 0.03 mg; vitamin C (6-palmitoyl-L-ascorbic acid), 17.5 mg; vitamin C, 100 mg; pantothenic acid (D-calcium pantothenate), 40

mg; choline chloride, 650 mg; niacin, 75 mg; folic acid, 2.5 mg; biotin, 0.4 mg; betaine, 650 mg; copper, 10 mg; iron, 50 mg; iodine, 1 mg; manganese, 35 mg; zinc, 100 mg; selenium, 0.3 mg. [b] Brocacel [c] LignoBond DD [d] Nutri-C [e] Solumul 484 [f] Fructomax

Source: van Rooijen *et al.*, (2013)

Table 7. Nutrients requirements for dogs (in percentage or amount per pound of food)

Nutrient	**Composition of meal or other dry type of dog food[1] (%)**	**Composition of canned or wet mixtures of dog foods[1] (%)**
Moisture	8-10	70-75
Protein-minimum for growth	18.0	6.0
Protein-minimum for maintenance	13.5	4.5
Carbohydrate-for growth	70.0	23.0
Carbohydrate-for maintenance	75.0	25.0
Fat	4.5	1.5
Calcium (minimum)	1.0	0.4
Phosphorus (minimum)	0.8	0.3
Sodium chloride	1.4	0.5
Potassium	0.8	0.3
Mg. per lb. of feed		
Iron	22	8.0
Copper	2.5	1.0
Cobalt	1.0	0.3
Magnesium	200	70
Manganese	2	0.7
Zinc	2	0.7

Iodine	0.5	0.2
Vitamin A	0.6[2]	0.2[2]
Vitamin D	0.003[2]	0.001[2]
Vitamin E (growth)	20[3]	7.0[3]
Vitamin B_{12}	0.01	0.004
Thiamine	0.3	0.1
Riboflavin	0.8	0.3
Pyridoxine	0.4	0.15
Pantothenic acid	0.9	0.4
Niacin	4.1	1.5
Choline	560	200

[1] Because of the increased amounts provided, a diet supplying the nutrients required for maintenance will also give those required for growth or reproduction, with the exception of protein. The composition provided will meet the nutrient needs of large dogs; little dogs, who eat proportionately more food, will be given more liberal portions.

[2] The weights shown are for pure crystalline vitamin A and vitamin D. These vitamins are typically provided in the form of fish liver oils, coupled with carrier oils or solids that dilute them for easy handling. Appropriate weight increases must be made to account for these dilutions. The corresponding weights equal to 1250 IU of vitamin A and 120 IU of vitamin D per pound of food. Carrotene may be able to replace some of the vitamin A. Carotene is roughly 60% as potent as vitamin A, hence higher doses are required. Each milligram of vitamin A corresponds to 3,000 units; each milligram of carotene corresponds to 1800 units.

[3] As alpha tocopherol or a physiologically active substance equal.

Source: National Research Council (USA), 1953

Table 8. Standard Ingredients and their analysed chemical composition for dog food production

Ingredients	Parameters	Chemical composition (%)	source
Wheat	Moisture	12-13	Oberoi *et al.*, 2007
		12.67	Kulkarni *et al.*, 2012
		10.58	Ikese *et al.*, 2012
		9.08	Ghodke, 2009

	Carbohydrate	72	Oberoi *et al.*, 2007
		74.40	Ghodke, 2009
		74.88	Kulkarni *et al.*, 2012
		84.34	Sulieman *et al.*, 2014
		80.76	Jaekel *et al.*, 2012
		69.26	Ikese *et al.*, 2012
	Crude protein	12.43	Ikese *et al.*, 2012
		8-13	Oberoi *et al.*, 2007
		13.36	Ghodke, 2009
		10.55	Kulkarni *et al.*, 2012
		13.19	Sulieman *et al.*, 2014
		15.41	Jaekel *et al.*, 2012
	Crude fat	1.5	Oberoi et al., 2007
		0.94	Kulkarni *et al.*, 2012
		2.01	Ghodke, 2009
		1.24	Sulieman *et al.*, 2014
		2.12	Jaekel *et al.*, 2012
		2.58	Ikese *et al.*, 2012
	Crude ash	0.94	Kulkarni *et al.*, 2012
		2.59	Ikese *et al.*, 2012
		1.15	Ghodke, 2009
		0.68	Sulieman *et al.*, 2014
	Soluble protein	0.1	Oberoi *et al.*, 2007
	Crude fibre	0.56	Sulieman *et al.*, 2014
		2.56	Ikese *et al.*, 2012
		12.00	Jaekel *et al.*, 2012
	Mineral salts	0.5	Oberoi *et al.*, 2007
	Calcium (mg/100g)	18	Kulkarni *et al.*, 2012
	Iron (mg/100g)	2.1	Kulkarni *et al.*, 2012
	Iron (ppm)	20	Sulieman *et al.*, 2014
	Phosphorus (mg/100)	107	Kulkarni *et al.*, 2012
Corn	Moisture	12.0	Mesfin & Shimelis

			(2013)
		7.16	Ape David *et al.*, 2016
		9.60	Sanchez-Madrigal *et al.*, 2017
		13.0	Nweke (2010)
		1.78	Fenta & Kumar (2018)
		11.38	Ambardekar (2004)
	Carbohydrate	74.5	Mesfin & Shimelis (2013)
		76.0	Nweke (2010)
		82.24	Fenta & Kumar (2018)
		75.23	Sanchez-Madrigal *et al.*, 2017
		77.46	Ape David *et al.*, 2016
	Crude protein	9.0	Mesfin & Shimelis (2013)
		7.0	Nweke (2010)
		7.88	Fenta & Kumar (2018)
		7.09	Sanchez-Madrigal *et al.*, 2014
		8.75	Ape David *et al.*, 2016
		7.00	Ambardekar (2004)
	Crude fat	3.4	Mesfin & Shimelis (2013)
		1.5	Nweke (2010)
		4.56	Fenta & Kumar (2018)
		4.44	Sanchez-Madrigal *et al.*, 2014
		2.40	Ape David *et al.*, 2016
		1.26	Ambardekar (2004)
	Crude ash	1.1	Mesfin & Shimelis (2013)
		1.0	Nweke (2010)

		1.40	Fenta & Kumar (2018)
		1.38	Sanchez-Madrigal *et al.*, 2014
		2.19	Ape David *et al.*, 2016
		0.55	Ambardekar (2004)
	Crude fibre	1.0	Mesfin & Shimelis (2013)
		2.26	Sanchez-Madrigal *et al.*, 2014
		2.14	Fenta & Kumar (2018)
		6.5	Nweke (2010)
		2.40	Ape David *et al.*, 2016
		2.77	Ambardekar (2004)
Chicken meal	Dry matter	90.14	Adewolu *et al.*, 2010
		90.9	Ambardekar (2004)
	Moisture	7.56	Olaniyic *et al.*, 2016
	Crude protein	56.62	Adewolu *et al.*, 2010
		45.50	Olaniyic *et al.*, 2016
		63.87	Ridwanudin & Sheen (2014)
		35.0	Oke *et al.*, 2016
	Crude fat	2.70	Adewolu *et al.*, 2010
		24.09	Olaniyic *et al.*, 2016
		13.92	Ridwanudin & Sheen (2014)
		22.0	Oke *et al.*, 2016
	Crude ash	3.42	Olaniyic *et al.*, 2016
		15.91	Ridwanudin & Sheen (2014)
		6.3	Oke *et al.*, 2016
	Crude fibre	5.84	Adewolu *et al.*, 2010
		2.15	Olaniyic *et al.*, 2016
Meat meal and	Moisture	8.40	Ambardekar (2004)

bone meal	Dry matter	93.4	Muir *et al.*, 2013
		93.64	Hayashi *et al.*, 1999
		91.3-96.2	Shirley & Parsons (2001)
		953.8 (g kg^{-1})	Al-Qazzaz *et al.*, (2016)
	Crude protein	50.3	Muir *et al.*, 2013
		55.79	Ambardekar (2004)
		33.70	Hayashi *et al.*, 1999
		30.6-54.4	Shirley & Parsons (2001)
		441.8 (g kg^{-1})	Al-Qazzaz *et al.*, (2016)
	Crude fat	6.0	Muir *et al.*, 2013
		8.99	Hayashi *et al.*, 1999
		217.8	Al-Qazzaz *et al.*, (2016)
		9.45	Ambardekar (2004)
	Minerals	45.45	Hayashi *et al.*, 1999
	Crude fibre	3.03	Ambardekar (2004)
	Crude ash	36.1	Muir *et al.*, 2013
		23.32	Ambardekar (2004)
		8.8-62.8	Shirley & Parsons (2001)
		164.9 (g kg^{-1})	Al-Qazzaz *et al.*, (2016)
Maize gluten feed	Moisture	11.0	Yigit *et al.*, (2012)
	Crude Protein	45.0	Yigit *et al.*, (2012)
	Crude fat	2.7	Yigit *et al.*, (2012)
	Crude ash	3.2	Yigit *et al.*, (2012)
Bloodmeal	Dry matter	95.52	Nahar *et al.*, (2000)
	Moisture	10.12	Opurum *et al.*, (2017)
		12.86	Ambardekar (2004)
	Crude Protein	88.54	Opurum *et al.*, (2017)
		84.63	Ambardekar (2004)

		80	Mandal, 2014
		83.12	Ewa *et al.*, (2017)
		90.02	Nahar *et al.*, (2000)
	Crude fat	1.14	Opurum *et al.*, (2017)
		0.08	Ambardekar (2004)
		1.0	Ewa *et al.*, (2017)
		1.0	Mandal, 2014
		1.40	Nahar *et al.*, (2000)
	Crude ash	4.34	Opurum *et al.*, (2017)
		6	Mandal, 2014
		5	Ewa *et al.*, (2017)
		2.36	Ambardekar (2004)
		6.48	Nahar *et al.*, (2000)
	Crude fibre	1.25	Opurum *et al.*, (2017)
		0.02	Ambardekar, 2004
		1	Mandal, 2014
		1.24	Nahar *et al.*, (2000)

The analysed chemical composition of standard ingredients used for the production of dog food (Table 8) cleary indicates that *Lumbriculus variegatus* (47.8-68%), *Hermetia illucens* (17.5-62.7%) and *Tenebrio molitor* (47-55.30%) can sufficiently fully or partially replace the protein ingredients in a model dog food such as chicken meal (35-63.87%), meat and bone meal (30.6-55.79%) as well as bloodmeal (80-90.02%)

Table 9. Essential amino acid requirements of dogs

Amino Acid	**% of dry matter**	**Mg/g N ***
Arginine	0.50	142.9
Histidine	0.18	51.4
Isoleucine	0.36	102.9
Leucine	0.58	165.7
Lysine	0.51	145.7
Methionine + cystine	0.39	111.4
Phenylalanine + tyrosine	0.72	205.7
Threonine	0.47	134.3
Tryptophan	0.15	42.9
Valine	0.39	111.4
Non-essential amino acid	6.26	1788.6
Calculated based on the diet being 22% protein and that the protein in the diet was 16% nitrogen		

Source: Brown (1989)

The efficiency at which amino acids are transformed into tissues depends on the protein quality, which also depends on the amino acid concentration in the pet food (Brown 1989). Table 9 indicates a list of essential amino acids required by dogs for growth and development. Essential amino acids are known as amino acids that cannot be synthesized by the body through metabolism (Lopez & Mohiuddin, 2020) and must be supplied externally in the pet diet. Amino acids are either essential or non-essential based on previous research, indicating that growth or nitrogen balance requires specific amino acids. However, there are sufficient alternative amino acids (Brown, 1989). All the nine essential amino acids can be derived by a single complete protein mainly obtained from animal-based sources except soy (Hoffman & Falvo, 2004).

2.4. Processing of insect meal

With reference to an article written by the International Platform of Insects for Food and Feed (IPIFF) (2019), the processing of insects for animal feed includes: slaughtering (heating or freezing) and post-slaughtering (drying and grinding). These measures are necessary not only to ensure safety but also to preserve the composition of the nutrient. Blanching, freezing, cooling, and drying are part of the slaughter process (Hong *et al.*, 2020). These processing factors enable insect larvae to be transported and stored for a long time (Hoback & Stanley, 2001). Many investigators have sought the best way to optimize both safety and nutritional benefit (Glendon & Stanton, 2000). Blanching before cooling or drying has been proposed, as blanching allows for the killing of vegetative cells and inhibits microbial development during storage (Larouche *et al.*, 2019). The high moisture content of insect larvae (approximately 68%) makes drying necessary after slaughtering (Hong *et al.*, 2020). This increased moisture level may induce microbiological spoilage and enzyme or non-enzyme degradation (Kröncke *et al.*, 2019). A moisture level of 4-5% is thus, recommended to minimize potential issues (Makkar *et al.*, 2014). The main drying methods used in drying include oven drying, vacuum drying, freezing, and microwave drying (Kröncke *et al.*, 2019; Vandeweyer *et al.*, 2017; Bubler *et al.*, 2016). Kröncke *et al.* (2019) showed that various kinds of drying did not lead to a vast diversity of *T. molitor* larvae's nutrition parameters. Insect larvae might undergo further processing stages before grinding, for example, defatting or hydrolysis (Figure 1).

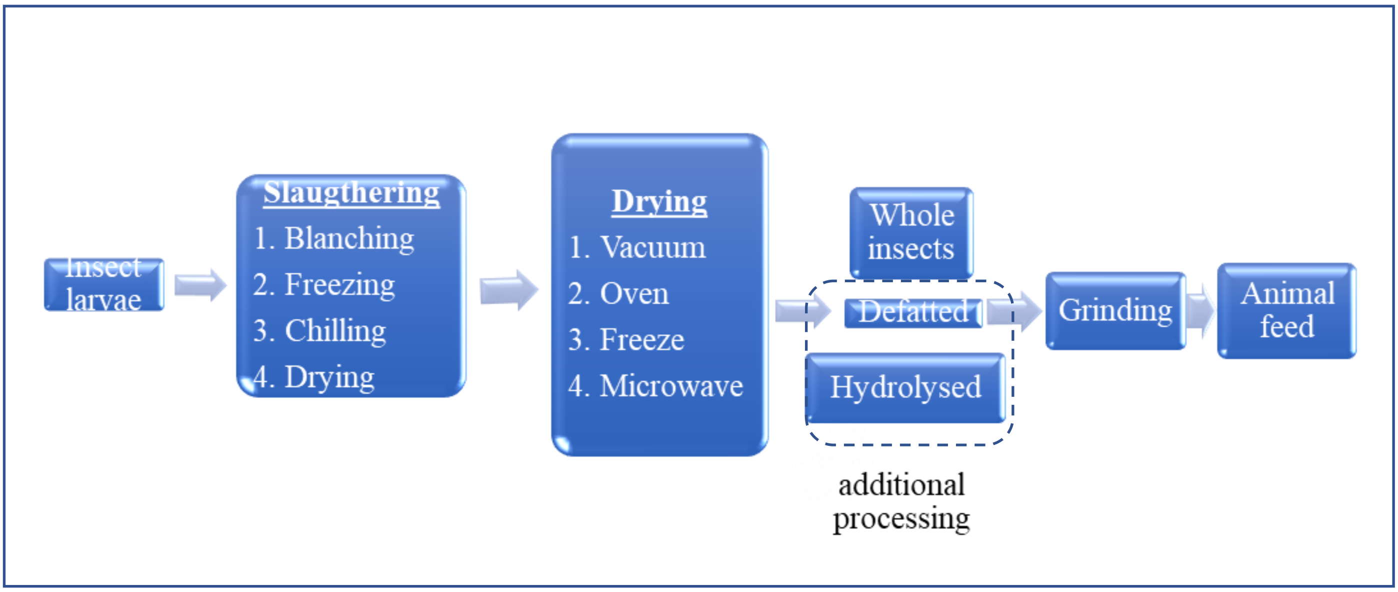

Figure 1- Production process of insect meal (Hong *et al.*, 2020)

Insect larvae are milled and incorporated into feed processing line either as a complete (full-fat) (Ramos-Elorduy *et al.*, 2002; Jin *et al.*, 2016; Ng *et al.*, 2001), defatted (Cho *et al.*, 2020; Ido *et al.*, 2019; Rema *et al.*, 2019; Ko *et al.*, 2020) or hydrolyzed feed component (Cho *et al.*, 2020). Defatting is essential for extended storage and processing (Hong *et al.*, 2020). The reason is that full-fat insect larvae have a high concentration of fat (25-35%) and fatty acids (10–30%) and are sensitive to fat oxidation during drying and storage (Ghosh *et al.*, 2017;

Lenaerts *et al.,* 2018). Defatting can be performed either by high-pressure (Thevenot, 2018) or organic solvent (Zhao *et al.,* 2016) and supercritical CO_2 (Laroche *et al.,* 2019; Purschke *et al.,* 2017). In a recent study, hydrolyzed *T. molitor* larvae have been added to pig diets to minimize potential anti-nutrients (Cho *et al.,* 2020). They found that *T. molitor* larvae hydrolyzed enhance ileal digestibility of pigs. *T. molitor larvae* were also conceivable with the evolution of the processing technology to extract and purify protein or fat (Bubler *et al.,* 2016; Zhao *et al.,* 2016). They have now being utilized as nutritional and functional dietary components for humans (Son, 2020). However, insect larvae can also use protein or fat removed for animal feeding, which must be investigated. Protein isolated from insect larvae appears to have functional characteristics (protein solubility, water and oil holding capacity, foaming, and emulsifying properties) affected by the processing time and temperature (Zhao *et al.,* 2016; Lee *et al.,* 2019). Consequently, appropriate, and effective processing parameters for insect larvae should be devised in the future for effective feed manufacturing (Hong *et al.,* 2020).

2.4.1. Protein

It is vital to realize that cereal grains cannot supply the amino acid balance needed to ensure healthy development and body maintenance when creating and processing animal feed via extrusion cooking alone (Nuss & Tanumihardjo, 2010). Protein components are therefore used to guarantee a complete nutritional diet (Kreb-Smith *et al.,* 2018). The protein content is generally between 25-70% of the recipe (Rokey *et al.,* 2010). Protein is sourced from either plants or animal origin (Bernier-Jean *et al.,* 2020).

Plant proteins commonly used include soy meal, wheat gluten, and maize gluten (Hardy, 2010). Plant proteins' structural and nutritional features contribute significantly to pet diets (Singh & Wakeling, 2007). Pet diets production cannot only utilize vegetable protein sources, as they do not supply all the primary amino acids (Rokey *et al.,* 2010). Proteins from animal origin commonly used include poultry by-products, fish meal, meat and bone meal, blood meal, and gelatine (Hicks & Verbeek, 2016).

2.4.2. Fat

Fats or lipids are an outstanding energy source in pet food (Tran & van der Poel, 2008). Fat levels may surpass 30% but are generally below 20% of the entire formula (Rokey *et al.*, 2010). Extrusion at low moistures (<20%) and high temperatures (>150°C) is very likely to lead to the formation of lipid/starch and lipid/protein complexes (Leonard *et al.*, 2020). In these conditions, free fatty acids and polar lipids are very reactive (Rokey *et al.*, 2010).

The added fat amount may change the product properties during extrusion processing (Singh *et al.*, 2007). The fat content and the fat source impact the expansion rate during extrusion (Tran & van der Poel, 2008). Indigenous fats used as an ingredient component are likely to have a lower expansion impact than refined fat (Cooper & Weber, 2012). For instance, a 15% fat formulation in which the fat is provided as part of that formulation through full fat canola has less influence on growth than the addition of pure canola oil to the same fat content in a product (Rokey *et al.*, 2010).

Heating fat sources at between 40 and 60°C before mixing with the rest of the formulation will limit temperature-dependent viscosity changes and help to cook the whole product (Rokey *et al.*, 2010). Tallows, chicken fat, vegetable oils, marine oils and different mixes from all sources are fat sources (Rokey *et al.*, 2010). Durable fats can be used for less fat migration to retail packaging during storage (with high melting points) (Rokey *et al.*, 2010).

2.4.3. Fibre

Calorie-reduced pet meals are becoming widespread in the market for fat or sedentary pets (Rokey *et al.*, 2010). The cellulose and hemicellulose are important in such because cellulose is formed from chains of glucose molecules linked together, but dogs and cats do not have enzymes that can break it down, thus it travels through the digestive tract pretty much unaltered diets (Rokey *et al.*, 2010). A dog or cat's digestive tract cannot digest or ferment cellulose, thus adding it to food might lower the calorie content, which may aid with weight control. Extrusion is seldom sufficiently severe for the seeming digestibility of dietary fibres (Rokey *et al.*, 2010). However, fibrous materials are significantly different in bulk densities and hydration characteristics than conventional ingredients (Rokey *et al.*, 2010).

The continuous carbohydrate matrix of the extruded product is disrupted to high amounts of fibrous components, which lead to rough appearance and excessive fineness (Rokey *et al.*, 2010). Depending on the kind, growth circumstances and milling characteristics of the grain, the starch of various fibrous components (e.g. wheat middles or rice bran) might vary between 16-40% (Rokey *et al.,* 2010). Changes in the extrusion process are the changing starch levels (Liu *et al.,* 2017).

As a linear polymer of N-acetyl-glucosamine units, chitin is embedded in a matrix of proteins in insect cuticles (Andersen *et al.,* 1995). When it comes to cuticle mechanics, proteins and sclerotization play an important role (Andersen *et al*., 1995). The quantity of chitin in *Hermetia illucens* and *Tenebrio molitor* larvae was determined to be 5.4 and 2.8 percent of dry matter (DM) respectively (Finke, 2013). There are chitinases that break down chitin in the human gut, as well as in mice (Boot *et al.,* 2005) and several bat species (Strobel *et al*., 2013). However, despite the fact that dogs are descended from the wolves, which have a low invertebrate diet (Bosch *et al*., 2015), their genome is also made up of chitinase protein-coding genes according to Bussink *et al.,* (2007). Cornelius et al., (1975) also found chitinolytic activity in extracts of the gastric mucosa of dogs, suggesting gene expression and secretion. It was shown that the digestion efficiency for this specific source was quite poor (Okamoto *et al*, 2001). A limited level of microbial chitinolytic activity was also seen in the dogs, which suggests that chitin is not fermentable fiber.

2.5. Minor ingredients

A category of minor components is crucial for vitamins. During heat treatment, each vitamin has its specific behavior, and some are unstable while stored. Moisture, pressure, shear and temperature impact the stability of vitamins during the cooking of extrusion. During extraction, fat-soluble vitamins like vitamins A, D, and E are relatively stable (Rokey *et al.,* 2010). The humidity level of the extrusion is the most important impact preserved in vitamins (Dar *et al.,* 2014). In general, higher levels of humidity provide more preserved vitamins (Ball, 1997).

2.6. Dog food production process

Commercial pet meals may be categorized into four main diet types: dry, semi-moist, moist, and snacks (Tran, 2008). The largest sector in the value and quantity of pet meals sold worldwide is dry pet food (Laxhuber, 1997). The reduction to a specified low level of the moisture content of pet meals offers ideal conditions for kibble coating (Tran, 2008).

Moreover, the minimal water level provides optimum protection during storage (additional moisture causes the development of microbes) and transit (costly for transport of water) (Medic *et al.*, 2012). Microbial growth can develop in wet meals if they are not adequately prepared or kept (Rawat, 2015).

Extrudates from raw materials, including fish, meat, grains, and other vegetable items, are generated in different forms and sizes in pet food production (Alam *et al.*, 2016). Raw materials can be treated in various ways before cooking, and automation can be utilized at different stages (preconditioning) (Fig. 2).

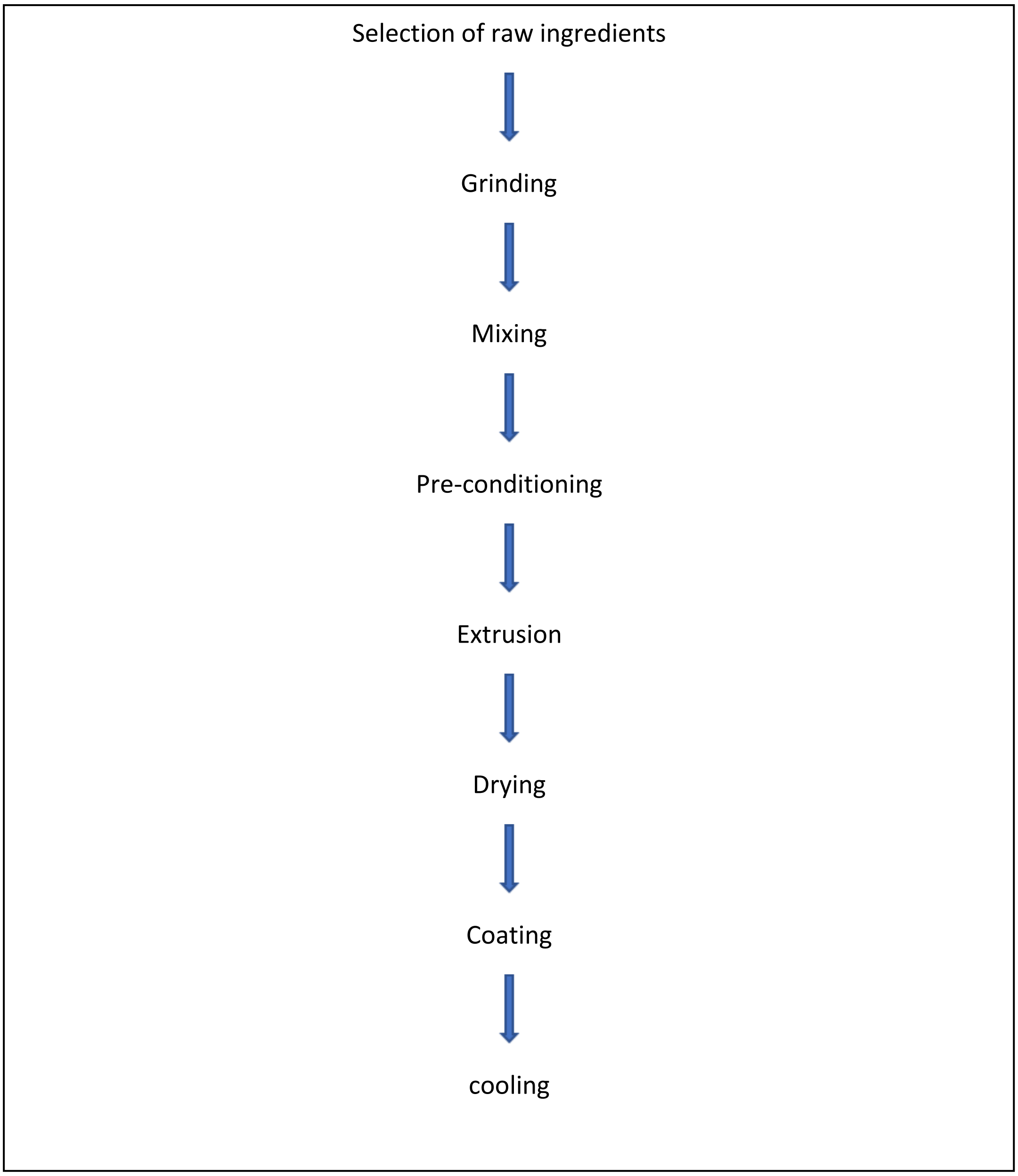

Figure 2- Production flow of dog food

The first stage is to combine different raw ingredients and water in a predetermined mixing ratio (Fu, 2008). Raw ingredient formulation, process equipment selection, and processing conditions are distinct control zones exerted in pet food extrusion cooking (Huber, 2000).

The selection of ingredients significantly influences the texture, standardization, extrudability, nutritional quality, and economic viability of finished products (Elabadi, 2018). Insects are usually ground with maize starch, oatmeal and pea fibre to prevent blocking as a result of the high-fat content in the insects, before the first mixing. After the first mixing, the macro ingredients are ground in a small Hammer mill (Bill bliss, horizontal, 18,5kW, USA) with a 0,6mm screen. A small K-Tron is used to feed the Hammer mill on all diets. After grinding, it is then mixed together with the other ingredients. The extrusion cooking technique may create various products within specific restrictions defined by a nutritionist (Sun *et al.,* 2018). The moistured granular or flour components are transformed into the dough during extrusion cooking of cereal grain and protein mixtures (Guy, 1994). The starchy ingredients gelatinize, which significantly increases moisture and the dough's viscosity (Lai & Kokini, 1991). Specific protein components can affect the characteristics of elasticity that characterize hydrated and developed sticky dough (Maache-Rezzoug *et al.*, 1998). Other protein sources, such as meat meal or fishmeal, which have poor protein solubility, may contribute less to sticky and stretchy functional characteristics (Day, 2011). Consistent particle size distribution improves all particles' even absorption of moisture; thus, grinding is done before mixing (Bolenz *et al.,* 2014).

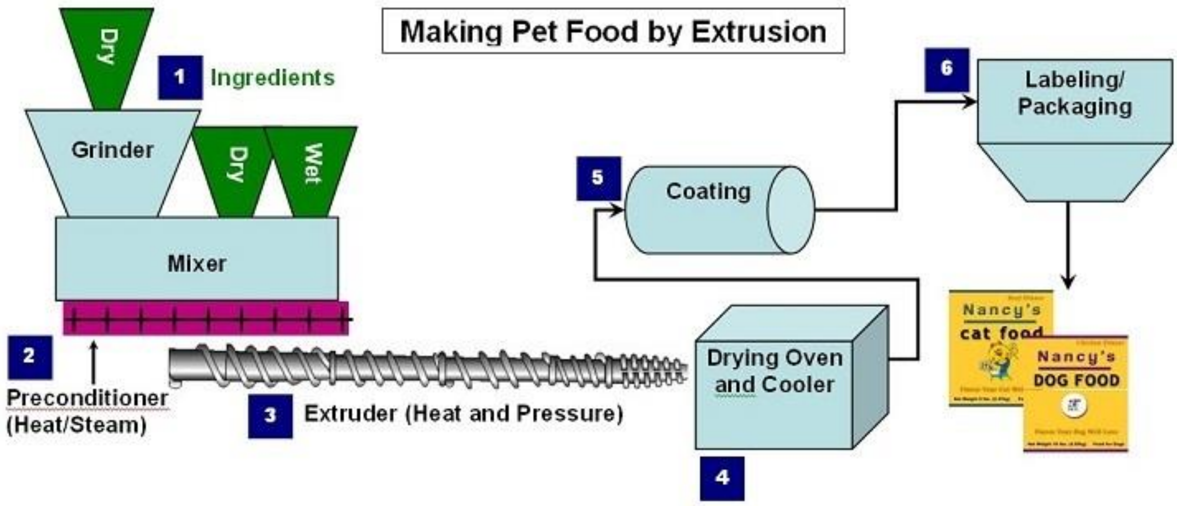

Figure 3- Production of dog food through extrusion (https://ipupster.com/how-is-dry-dog-food-made/)

The homogeneity of the mixture before extrusion guarantees proper and consistent cooking of each particle (Cheftel *et al.*, 1992). This improves the look and taste of pet food (Phillips & Finley, 1989). The ingredients are then treated by an extruder, and for that purpose, different extrusion methods and device types were created (Sørensen, 2012). Extrudates are generally dried after extrusion to ensure consistency and storage (Jamora & Rhee, 2002). Extrudates are frequently coated with a coating to add spice or aroma to the extrudates (Leonard *et al.,* 2020).

Moreover, energy-producing ingredients are occasionally added like lipids, and the goal is sometimes to enhance the structure of the surface (Bocquier & Gonzalez-Garcia, 2010). Fat and oil suspensions are commonly utilized as a coating for pet food manufacture (Gibbs, 1999). During the coating process, fat must be absorbed as quickly and thoroughly as possible before cooling over hot, dry extrudates (Riaz, 2000). Depending on the product and ingredients used for coating, a further drying or chilling stage may be necessary (Desai & Jin, 2005). Food and feed extruders offer control over machine settings and set up to get a mix of different process characteristics (Moscicki & van Zuilichem, 2011). The process factors impact the effect on the product: selective processing can be used to optimize the denaturation of proteins, gelatination of starch, and modify the fat globules (Chandrapala *et al.,* 2012). Moisture content assessment is one of the most significant factors in processing (Rathod & Annapure, 2016). The full moisture penetration of the component particles leads to a heat transfer that can lead to consistent gelatinization of starch and a cooked product with a high-fat level (De Pilli & Alessandrino, 2020).

Extrudate physical features reflect the processing efficiency; thus, the appropriateness of extrusion ingredients can also be derived from this (Patil *et al.,* 2005). Pet food undergoes chemical and physical changes during extrusion, which modify the physical and nutritional quality of the product (Kvamme and Phillips, 2003). The modifications can include disulphate bridges forming proteins and Maillard products, resulting from an interaction between alkaline amino acids and sugar reduction (Mauron, 1990). Extrusion cooking splits oil globules to expand oil easily (Lin *et al.,* 1998). It also textured protein and caused protein denaturation and starch gelatinization (Lin *et al*., 1997). Protein changes may digestibly increase the digestion of dietary proteins (MacLean *et al*., 1983; Coulter and Lorenz, 1991). The quality of protein is enhanced in this regard (Wille-Reece *et al.,* 2005). Extrusion processing affects the dietary features of extruded products by changing the availability to metabolizable proteins, carbohydrates, lipids, and vitamins (Alam *et al.,* 2016). Protein denaturation, structural modification, lipid oxidation, and reactions, such as Maillard reactions, may change the nutritional characteristics of extrudates (Asp and Björck, 1983).

Furthermore, extrusion cooking involves a temperature and pressure that inactivates natural toxins (mycotoxins, glycoalkaloids, and allergies) and nutritionally active components (trypsin inhibitors, gossypol, etc.) (Brimer, 2011). It also removes microorganisms that contaminate the products (Harper and Jansen, 1981). Extrusion is, therefore, a complicated process that involves the interconnection between process and product factors (Ariff *et al.*, 2012).

According to Nokland, (2019), insect feed with a high lipid content enhanced the amount of fat in the mash prior to extrusion. In accordance with Hansen *et al.* (2011) and Lin *et al*. (1997), this resulted to enhanced lubrification during extrusion. Also according to Nokland, (2019) experiment, higher screw speed compensated for increased lubrification in his experiment, primarily to achieve equivalent physical pellet qualities between diets. However, despite the increased screw speed, the torque, temperature, die pressure, and SME all reduced as the inclusion amount of insect meal or insect paste was raised (Nokland, 2019). To compensate for higher amounts of lipid in the mash during extrusion, Hansen *et al.* (2011) reduced the amount of liquid added during extrusion. Insect meal inclusions demonstrate the impact of greater lipid addition in animal diet (Nokland, 2019). Reduce torque, temperature, die pressure, and SME when insect meal or insect paste was used in higher concentrations was found to be beneficial in diet production (Nokland, 2019). Mash with less fat enhance SME (Aslaksen *et al.*, 2007). Screw speed, throughput, and flowability likely impact residence time and perhaps cooking time (Riaz, 2007). Due to increased transporting capabilities of screws, a faster screw speed would result in a reduction of residence time (Puaux *et al*., 2000).

Resistance to flow, resistance given by die, screw, or mash composition, and binding (viscosity and lubrication), and finally the quantity of backfilling in the extruder barrel, would all impact throughput in an extruder. Lubrification reduces torque, which is dependent on flowability (friction), barrel filling rate (backfilling), and viscosity (Forte & Young, 2016) thus, In terms of die pressure and torque, this would result in less backfilling (less residence time and cooking) (Nokland, 2019).

In the SME computation, residence time is solely taken into account through torque (Nokland, 2019). In high-fat diets, this computation of mechanical energy cooking of the ingredient may not be accurate since the residence period would be greatly affected (Nokland, 2019). The SME grows linearly as the screw speed increases (Riaz, 2007), yet the residence time of the ingredient decreases as the screw speed increases (Nokland, 2019). Extrusion of a high lipid mash would be less influenced by mechanical energy (shear forces) due to the reduced friction thus, cooking time would be reduced, as would time spent in storage after processing (Nokland, 2019).

Temperature, screw speed, and mash composition would also impact torque as a function of viscosity, as mash often behaves as a shear-thinning liquid when entering a melt stage (Forte & Young, 2016). During extrusion, this might potentially have an impact on the torque % measured, as well as the increased quantity of lipid in the mash (Nokland, 2019).

A thorough review of the literature on the extrusion cooking method of dogs' diet suggests that the extrusion effects were not adequately investigated and documented regarding the interactions between dietary components and extrusion phases, although they are critical characteristics (agglomeration and drying) (Tran, 2008).

Protein is considered as one of the main nutrients in dog nutrition (Daumas *et al.,* 2014). Animals utilize cereals as an essential nutritional ingredient, and hence it is vital during extrusion to keep or even increase cereals' protein quality (Arendt & Zannini, 2013). Amino acid composition, availability, and digestibility define a protein's nutritional quality (Paquet *et al.*, 1987; Philips & Finley, 1989). Lysine is frequently the most limiting amino acid in cereals (Boisen *et al.*, 2000). Due to its highly reactive free amino group, lysine can react with other nutrients, for example, sugar, relatively fast (Mehta & Deeth, 2016). This quickly changes or damages lysine (under extreme circumstances) and is occasionally unavailable for metabolism (Hurrell and Carpenter, 1981). Changes in food, feed, or reactive lysine (Lysine with a free amino group) may prove protein damage during extrusion or storage (Tran, 2008).

2.7. Changes in rheological properties of insect meal during processing

Recent studies have shown that insect proteins are included in pasta, bread, and other bakery produce (Gravel & Doyen, 2020). *Tenebrio molitor* flour (10-20%) was added to cereal snacks by Azzollini *et al.* (2018), and its nutritional, physical, and microstructural characteristics were assessed by the various treatment methods employed during manufacture (Gravel & Doyen, 2020). Gravel & Doyen, (2020) research demonstrated an increase in the protein content and digestibility of the snack in the integration of insect flour. In total, 10% of the *Tenebrio molitor* meal had cereal snacks that showed similar texture to their insectless counterparts, but 20% of the *Tenebrio molitor* meal had poor texture (Gravel & Doyen, 2020). Therefore, choosing a processing technique properly is an essential stage in the formulation of protein-rich food since the sensory and nutritional characteristics might change appropriately (Azzollini *et al.*, 2018).

2.8. Challenges with the use of insects as a protein source for dog food

Insects edible are regarded as one of the most fascinating sources of future protein because a big part of the global population lives with food insecurity (Gravel & Doyen, 2020). Acceptance of insect-containing foods is one of the major problems (Jensen & Lieberoth, 2019). Furthermore, numerous other obstacles occur to mainstream entomophagy in western societies (Jensen & Lieberoth, 2019). Some of them are also summarized here.

2.8.1 Consumer acceptance

Westerners are hesitant to try edible insects and to integrate them into cuisine (Gere *et al.,* 2017; Vanhonacker *et al.,* 2013; Verbeke, 2015) Moreover, authors discovered that disgust was a substantial factor (Jensen & Lieberoth, 2019). They indicated that enhancing the whole experience of entomophagy (taste, texture, presentation) would be significantly more efficient to make insects better accepted as a protein alternative rather than increasing their familiarity to lessen food neophobia (La Barbera *et al.,* 2018). Some studies have profiled customers who will try to eat foodstuffs based on insects (Jensen & Lieberoth, 2019). This research showed that men are more likely than women to use insects as protein substitutes because they are more adventurous eaters (Schosler, *et al.,* 2012; Verbeke, 2015). The studies also recognize that the relevance of producing goods with processed insects is underlined by insect-based foodstuffs that are not apparent to the consumer (Schosler, *et al.,* 2012; Gere *et al*., 2017).

In all, most research has shown that western consumers are not yet prepared to eat insects and that they are far less likely to take insects into account in regular meals (Amato, 2017; Piha *et al.,* 2018; Schosler *et al.,* 2012; Vanhonacker *et al.,* 2013; Verbeke, 2015).

2.8.2. Economy

As already noted, most insects are quite cheap for farming as they can be cultivated over short periods, with relatively little space, and require minimum feeding and care, making the most economically cost-efficient processing (Jensen & Lieberoth, 2019). The drying techniques utilized in laboratory investigations, such as freeze-drying, supercritical CO_2 drying, and microwave drying, are too expensive to apply commercially (Veldkamp *et al.*, 2012). Thus, at each stage of the process, the choice of a technique might require a compromise between profitability and functional properties (Jensen & Lieberoth, 2019).

2.8.3. Environment

Initially, van Huis *al.* (2013) drew more comprehensive attention to insects as a novel source of protein in agriculture and food science. Since insects are cold-blooded, they don't have to use the same amount of energy to maintain their inner temperature (Veldkamp *et al.,* 2012). This also has an impact on their emission and conversion rate of greenhouse gases (Jensen & Lieberoth, 2019). Oonincx *et al.* (2010) assessed greenhouse gas emission levels (GHG) of five distinct insect species and determined that only 4 out of the 5 produces 1% of ruminant GHG. The authors also tested their ammonia emission levels, reporting that all results were below traditional animals (Oonincx *et al*., 2010). The same authors assessed four distinct kinds of insects' feed conversion ratio (FCR) (Jensen & Lieberoth, 2019). Among these four animals, only *Blaptica dubia* and *Hermetia illucens* converted feed effectively than traditional animals (Jensen & Lieberoth, 2019). In contrast, *Tenebrio molitor* and *Acheta domesticus* have FCR equal to pigs and poultry, depending on their diets (Oonincx *et al.*, 2015).

The life cycle assessment (LCA) still gives a more comprehensive environmental review of a food source across the whole supply line (van Huis & Oonincx, 2017). Few LCAs for insects have been published (Jensen & Lieberoth, 2019). All six LCA studies available for food and feed insects were revised by Halloran *et al.* (2016). They found it necessary to develop precise standards for future experiments and provide a clear insect evaluation as an alternative sustainable food (Jensen & Lieberoth, 2019). The authors argued that the varied methods resulted in incomparable data and different conclusions according to each study's functional unit and global objective (Halloran *et al.*, 2016). However, Smetana *et al.* (2019) conducted an experiment on *Hermetia illucens* regarding LCA and determined that insect protein concentrate was less environmentally friendly. Compared with animal protein concentrates, the impact has a more significant environmental effect than plant protein components (Smetana *et al.*, 2019). It is evident that LCA for food and feed insects are in their infancy, and thus, further research should be conducted in the future to prevent a problem in the edible area of insects.

2.8.4. Safety

It has been centries since the introduction of insect-based, comprehensive pet meals on the market and health concerns have not been documented yet (Beynen, 2018). Studies that lasted 28 (12-14) or 42 days (Kroger *et al.*, 2017), but did not assess clinical chemistry, found that meal worm and black soldier fly larvae-containing diets did not adversely impair the apparent health of animals (Beynen, 2018). There were no harmful effects with a dosage of 2.5 g cricket powder/kg bodyweight given to dogs (Ryu *et al.*, 2016). The long-term effects of insect eating on health are unclear and it is possible that insects carry harmful substances that they have created or acquired from their food sources (Beynen, 2018).

2.8.5. Allergies

The allergenicity of edible insects must also be addressed quickly (Jensen & Lieberoth, 2019). Cross-allergic responses to crustaceans and household dust mite proteins were linked with insects (Pali-Scholl *et al*., 2019; Ribeiro *et al.*,2018). The comprehensive analysis by Ribeiro *et al.* (2018) shows that all investigations of the littoral revealed tropomyosin and arginine kinase as the primary factors behind the allergic response to cross-reactivity and co-sensitivity between crustaceans, domestic dust mites, and dietary insects. This will make patients more likely to be allergic to edible insects or home dust mites allergies (Ribeiro *et al.*, 2018). This assertion might be confirmed clinically by a few investigations (Jensen & Lieberoth, 2019). Broekman *et al.* (2017) showed that 13 of 15 individuals who were confirming shrimp allergy were also allergic in various respects to mealworm (*Tenebrio molitor*) (Broekman *et al*., 2017). The same authors presented other insects to the participants in a further study (*Zophobas morio, Alphitobius diaperinus, Galleria mellonella, Hermetia illucens, Acheta domesticus* and *Locusta migratoria migratoria*) and concluded that such shrimp-allergic patients were not just allergic to *Tenebrio molitor*, but to other insects as well (Broekman *et al.*, 2017). Additional research has examined alternative means of eliminating or decreasing insect allergens, such as heat treatment and enzyme hydrolysis, depending on the insect being studied, thermal treatment, and the enzyme employed (Jensen & Lieberoth, 2019). Allergies to food can lead to skin problems in dogs and cats (Premrov *et al.,* 2021). Estimates place their incidence at around 5% for dogs' overall dermatological problems and up to 25% for their allergic skin disorders (Matricoti & Noli, 2018). Atopic dermatitis (AD) in dogs is characterized by a pruritic erythematous dermatitis of the face, ear canals and other parts of the body, such as the axillae, groin, and feet (Premrov *et al.,* 2021). Allergies to foods may only be ruled out in cases of AD, when clinical symptoms are present all year round (Premrov *et al.,* 2021). Food-induced canine AD is characterized by gastrointestinal symptoms such as diarrhoea, vomiting, tenesmus, soft stools, flatulence, and an increased number of bowel movements (Premrov *et al.,* 2021). There may also be respiratory symptoms (Hensel *et al.,* 2015). Primary sensitization or cross-reactivity with another allergen may cause an allergic reaction after eating insects (de Gier & Verhoeckx, 2018). When Immunoglobulin E (IgE) antibodies identify and bind comparable allergenic chemicals, the immune system responds (Premrov *et al.,* 2021). IgE cross-reactivity occurs often between allergens in closely related species or evolutionary highly conserved compounds present in distinct species and pan-allergens are a class of such molecules (Premrov *et al.,* 2021). Because of the potential for allergic cross-reactivity to new foods, cross-reactivity is crucial (Popescu, 2015). The skin condition known as atopic dermatitis (AD) in dogs is a chronic and recurring inflammatory and pruritic allergic skin disease that is typically related with the development of IgE antibodies against environmental and/or dietary allergens and approximately, 3 to 15% of the dog population is affected by this disease (Premrov *et al.,* 2021). In an experiment conducted by Premrov *et al.,* (2021) involving two groups of dogs (CH and CA), cross-reactivity of canine sera with mealworm proteins 34–55 kDa was detected in 75% of the mitespecific IgE-positive CH dogs and in 57% of mitespecific IgE-positive CA dogs. Cross-reactivity with mealworm proteins was slightly greater (71%) in mite-specific IgE-negative CA dog sera, but there was no cross-reactivity with mealworm protein extracts in CH dogs' sera. (Premrov *et al.,* 2021). There was cross-reactivity between the 14 kDa mealworm extract protein and mite-specific IgEpositive antibodies (Premrov *et al.,* 2021). CH dog sera was 62% and CA canine sera was 79% (Premrov *et al.,* 2021). The cross-reactivity of their sera with mealworm protein, clinical symptoms of allergy, and the presence of IgEs against storage mites *T. putrescentiae* and *A. siro* ($p > 0.05$) in their sera were not statistically significant (Premrov *et al.,* 2021). If a protein shares more than 35% similarity with a recognized allergy within a window of 80 amino acids or greater, it is deemed possibly allergenic in Allermatch^{TM}. But this web tool was designed to predict food allergies in humans (Premrov *et al.,* 2021). Nine of the discovered mealworm proteins were, nevertheless, deemed to be allergenic (Premrov *et al.,* 2021). Their entire sequences match recognized allergens in the Allermatch^{TM} database by 30–96% (Premrov *et al.,* 2021). Also, they found a significant degree of similarity between the two allergenic tropomyosins from distinct insect types thus, Tropomyosin from *T. molitor* has a greater identity (96%) than that from *Zophobas atratus*, which only has a partial sequence available. It has been determined that mealworm larvae's tropomyosin is a significant food allergy in humans (Klueber *et al.,* 2020) as well as perhaps in canines (Premrov *et al.,* 2021). When combined with chitin, a key component of the insect's exoskeleton, cuticle proteins provide insects their exterior covering (Leni *et al.,* 2020). The allergic *T. molitor* proteins apolipophorin-III, larval cuticular protein, and hemolymph protein were molecularly modeled and shown to have structures comparable to pollen and fruit allergens (Barre *et al.,* 2019).

When it comes to alkaline mealworm extract, alpha amylase is a well-known allergen for a number of different kinds of mites (Premrov *et al.,* 2021). Due to their varied degree of sequence similarity across mites, insects, and mammals (van Broekhoven *et al.,* 2016), alpha-amylases may operate as IgE-binding allergens capable of causing cross-reaction (Premrov *et al.,* 2021). Die Allergenicity of Feed is known to be affected by processing (heat or pressure) and/or hydrolysis (digestion) of food allergen proteins (Premrov *et al.,* 2021). If the binding epitopes are altered, the binding capability of IgE is reduced (de Gier & Verhoeckx, 2018; Olivry *et al.,* 2017). Also, according to Premrov *et al.,*(2021) protein extracted from *T. molitor*, contain many IgE-reactive proteins that have previously been reported to cross-react with IgEs from human sera sensitized to crustaceans or house dust mites. Therefore, caution should be used while utilizing mealworm larvae as an alternate protein source in the case of dogs sensitive to mites. However, literature on the allergicity of *Lumbriculus variegatus* and *Hermetia illucens* relating to dogs is limited, thus, more research work should be conducted in this apect. Once more, it is important to emphasize the absence of scientific documentation on the safety of entomophagy and remedies to these problems (Jensen & Lieberoth, 2019). As insects become a trend in the food market, food researchers have to look into the issue quickly (Jensen & Lieberoth, 2019).

2.9. Conclusion

Lumbriculus variegatus, *Hermetia illucens*, and *Tenebrio molitor* have shown quiet significant potential to used as an alternative protein sources for dog food production. Currently, the use of *Hermetia illucens*, and *Tenebrio molitor* as protein sources as been approved and being used commercially for the production of dog food. Although *Lumbriculus variegatus* as the nutritional potential to be used commercially for dog food production, no commercial product was found. From literature, the focused insect was used mainly for the production of the feed for monogastric and fish and they showed good results. However, usage of these insects comes with few limitations in terms of chitin indigestibity and also heavy metals found in some of them especially *Tenebrio molitor.* Furthermore, a more sustainable way of producing these insects commercially should be looked into in other to make it less costly for dog food producers. These insects could be a sustainable protein source for the production of dog food as its nutritional content match up to most of the protein ingredients used in the production of dog food as well as maintaining biosafety. Lastly, more research need to be conducted on the technological effect on these insects as they pass through the production process.

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