

**NUTRITIONAL VALUE OF LOCAL FEEDSTUFFS FOR SWINE
IN HAWAI'I**

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Nutritional value of local feedstuffs for swine in Hawaii

Abstract

Commercial swine diets are composed mainly of ingredients such as corn, wheat and soybean to meet energy and protein requirements. Market availability of these ingredients is variable due to limited production as well as competition among food, feed and fuel. Hence, in order to assure sustainability of swine production in places like Hawaii, alternative feeding system need to be studied, developed and extended. Some agricultural products like purple sweet potato(PSP), okinawan sweet potato (OSP), cassava and taro and some agricultural co-products like macadamia nut cake (MNC), okara, wheat millrun (WMR) and barley brewers grain (BBG), are available locally which could together provide basis for producing more affordable locally manufactured feeds. However, limited information available on nutritional value and digestibility of these potential feedstuffs, limits their use in routine swine feed formulation.

The agricultural products and agricultural co-products were obtained from local market to determine nutrient profile and in vitro digestibility. Among the agricultural products, crude protein (CP) content was highest in taro (8.8%) and lowest in cassava (3.7%). Ether extract (EE) was highest in PSP (2.7%) and lowest in cassava (1.0%). Acid detergent fiber (ADF) content was highest in taro (10.3%) and lowest in PSP (5.6%). Neutral detergent fiber (NDF) content was highest in taro (11.4%) and lowest in PSP (7.9%). The starch content was highest in cassava (60.8%) and lowest in taro (38.4%). Gross energy was highest in taro (4333 Kcal/kg) and lowest in PSP (4134). Among the agricultural co-products. Crude protein content was highest in MNC (25.5%) and lowest in BBG (11.7%). Ether extract content was highest in okara (13.3%) and lowest in BBG (1.7%). The ADF content was highest in MNC (28.0%) and lowest in okara (19.7%). The NDF content was highest in WMR (42.0%) and lowest in okara (31.0%). The

MNC had 2.8 and 0.2%, linoleic acid and linolenic acid content, respectively, lysine concentration was 0.7%. In vitro dry matter digestibility was significantly higher ($P < 0.001$) in PSP (86.8%) than taro (70.3%). In vitro gross energy digestibility was significantly higher ($P < 0.001$) in PSP (87.5%) than taro (64.9%). In vitro crude protein digestibility was significantly higher ($P < 0.05$) in PSP (76.3%) and cassava (74.4%) than taro (63.1%) and OSP (63.1%). Among agricultural co-products, in vitro dry matter digestibility among co-products was significantly higher ($P < 0.05$) in MNC (75.71%) and okara (74.10%) than BBG (61.33%).

Agricultural products can be used as partial substitute of common energy ingredients in pig diets, especially for subsistent farming system where agricultural products are grown and are widely available whereas agricultural co-products can also be used to replace traditional feed ingredients to some extent and can serve as potential source of protein.

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Chapter 1: Literature Review

1. Introduction

1.1 Status of swine industry in Hawaii

Pigs are very important part of Hawaii and Pacific island culture. Pork and other farm products have very high value, not only for *luaus*; a Hawaiian party or feast especially accompanied by entertainment, but also in feeding *Ohana* (family in Hawaiian language). The price of local pork is much higher in Hawaii than on mainland USA, so is the demand especially for very young pigs and sows for *kalua pork*; which is a whole pig baked in an *imu* (an underground oven) until the meat is very tender.

Swine farms in Hawaii are mainly small family farms with limited land and other resources. Farmers depend heavily on family labor. Farmers generally do not regard each other as their competitors; however, the swine industry in Hawaii has to compete with the large scale and cost-efficient producers from the mainland US.

Table 1. Total number of farms and pigs in Hawaii (USDA, 2012)

Year	Number of farms	Total number of pigs
1997	190	38,066
2002	158	33,231
2007	148	20,569
2012	131	12,529

There are about 66.3 million pigs in the USA, where the number of average pigs per litter is 10.14 (USDA, 2013). But, there are only 11,500 pigs in Hawaii (USDA, 2013), which is not adequate to meet the demand of pork in local market. Though the demand of pigs is very high, the supply does not come anywhere close to the demand. The trend has been the decrease in number of pig farms as well as the total population of pigs (Table 1). Number of pig farms decreased from 190 in year 1997 to 131 in year 2012, while total number of pigs decreased from approximately 38,000 in 1997 to 12,529 in 2012. Although swine breeds with high genetic potential are available locally, their population is too small to meet the local demand in Hawaii.

Table 2. Total percentage of pigs being slaughtered and imported (Zaleski, 2007; USDA, 2012)

Year	Total number of pigs slaughtered	Total number of pigs imported	Total % of pigs imported
2007	15,519	10,442	67
2012	14,200	11,700	82

Out of 15,519 pigs slaughtered in Hawaii, 10,442 were imported from mainland USA, i.e. almost 67% of slaughtered pigs in Hawaii were imported (Zaleski, 2007). While in 2012, out of 14,200 slaughtered pigs in Hawaii, 11,700 were imported live from mainland USA (USDA, 2012). It means that the import percentage increased to 82% just in five years, while locally grown pigs was less than 20% of total slaughtered.

Table 3. Number of sows, pigs per litter and gross income from hog sale (USDA, 2013)

Year	No of farrowing sow	Pigs per litter	Gross income from Hog sale, '000 USD
2008	2,800	7.14	3,546
2009	2,600	7.31	3,198
2010	2700	7.22	4,015
2011	2500	7.40	2,973
2012	2640	7.01	2,846
2013	2700	6.93	2,693

Since 2011, number of pigs born per litter has been constantly decreasing but there was slight increase in the number of farrowing sows, which increased by 4.0 % in 2012 and by 2.3 % in 2013.

The reason for this decreasing trend in swine production and overall swine industry is mainly attributed to high production costs, especially feed costs, limited availability of land, and competition with pigs imported live from mainland USA. Increased concerns for the environment have raised serious concern for all those who are taking part in pig rearing as well as sustainability of the swine industry. Of all these, the major problem that the farmers are facing is the feed cost. One of the worst things about living in an island like Hawaii being located in the middle of Pacific Ocean, approximately 2300 miles away from mainland US, is that whatever is imported has to travel a long distance before it arrives to the destination. Hence, the transportation cost will be very high in importing bulky items, irrespective of whether they are

feed ingredients to formulate feed or compound feed. The transportation costs add up to the total feed cost which is an additional burden to the farmers. No local grain-based diet is produced in Hawaii to feed the swine, and about 7100 tons of compound feed, worth more than \$5 million are imported annually (Ostrowski, 2011). In recent years, there has been a sharp increase in the price of internationally traded feed ingredients. In Hawaii, most of the commercial feed ingredients or compound feeds are shipped from the mainland US.

Commercial swine diets are composed mainly of ingredients such as corn, wheat and soybean to meet energy and protein requirements. Energy and protein are the most expensive components of swine feeds, thus the increase in cost of energy/protein-yielding feedstuffs will increase the cost of swine production. Corn, wheat and soybean are the most widely used feedstuffs in monogastric animals' diets. The price of corn has soared. This is because corn is used for production of ethanol (biofuel), thereby increasing the demand for corn by the ethanol industry (Tyner and Taheripour, 2007). Soybean is the most widely fed source of protein. The price of soybean meal (SBM) has soared as the land is being used to grow corn instead of soybean (Schmit et al., 2009; Avalos, 2014). Last 7 years resulted in increase in price of corn and soybeans more than double. Moreover, market availability of these ingredients is variable and the costs of these ingredients are expected to increase continuously due to limited production as well as competition among food (human), feed (livestock), and more recently, also for fuel. Therefore, it is very important to explore and evaluate alternatives to expensive energy and protein sources to combat high feed costs.

Potential alternative feedstuffs are available and can fulfill the nutritional requirements of livestock (FAO, 1997) and reduce feed costs (Woyengo et al., 2014). Several alternative feedstuffs, including cassava (Gomez, 1991), roots, bananas, organic waste (FAO, 1997),

tropical fruits, leaves and tubers (Leterme, 2006), taro (Buntha et al., 2008), novel grains and pulses (Beltranena and Zijlstra, 2007), oilseed cakes (Seneviratne et al., 2010), barley and oats (Jha et al., 2010) and wheat millrun (Norley et al., 2007; Jha et al., 2012a,b) have been evaluated and used successfully in swine diets elsewhere in the world. However, agro-climatic conditions and farming system largely differ from location to location which influence the nutritional profile of feedstuffs and their utilization in animals.

In order to assure sustainability of swine production in areas like Hawaii and other Pacific islands, where traditional feed ingredients cannot be grown or allocated solely to animal feeding, alternative feeding systems need to be studied, developed and extended. Some agricultural products which might serve as a source of energy like purple sweet potato (PSP), okinawan sweet potato (OSP), cassava, taro, as well as some agriculture byproducts which might serve as a source of protein like macadamia nut cake, okara and some agricultural co-products like wheat millrun and barley brewers grain, are available locally. These agricultural products/agricultural co-products could together provide basis for producing more affordable locally manufactured feeds. However, there is little or no information available on nutritional value and digestibility of these potential feedstuffs, which limits their use in routine swine feed formulation. Thus, detailed information on the nutritional value of these potential local feedstuffs is warranted before considering them for sustainable swine nutrition planning in Hawaii. Moreover, disposal of these byproducts results in economic loss and potential environmental impacts in Hawaii each year. Identification and information on nutrient utilization of potential local feedstuffs will not only improve the vigor of the swine industry through a reduction in feed cost, but also enhance the environmental sustainability by reducing waste disposal.

1.2 Nutrient requirement of pigs

1.2.1 Energy requirement

Swine production potential can be maximized only if the diet offered to them constitutes all the specific nutrients required by the animal. Above all, the most important factor affecting production potential is the energy intake (Harrell et al., 1993). Lactose is the major source of energy to piglets before they are weaned, as they are on liquid diet or milk diet. Once they are weaned, their major source of energy is changed from lactose to starch. Relative growth capacity of pigs is higher when the pigs are young and it reduces as they grow older, while feed intake capacity is lower when they are young and it increases as they grow old. Younger pigs require higher amount of energy. As they consume less, nutrient density in the feed should be high i.e. the diet should contain higher amount of energy. After weaning, the diet of piglets changes from sow milk to dry feed. Despite supplying a highly digestible diet, post-weaning stress also results in decrease in consumption of feed. In other words, it results in consumption of insufficient amount of feed, which does not meet the energy requirement needed by the weaned pigs (Pluske et al., 2002). This decrease in feed intake can result in digestive disorder, resulting in decreased body weight and increased mortality (Pluske et al., 2002). To help weaned piglets develop, the post-weaning diet should focus on increasing feed intake, and that intake should be increased early, which helps in the development of digestive tract (Cranwell et al., 1997). Though it can be expensive to offer a highly digestible diet to post-weaning piglets, such diets help lower the potential risk of diarrhea caused by the abrupt change of diet from liquid to solid form. Their capacity for feed intake increases with age; hence the energy content of diet is reduced.

Sows in the gestation period require relatively low amount of energy, and are still capable of eating more than what they actually need. As they consume more and require less than lactating sows, they are offered feed containing relatively lower amount of energy and their diet is also restricted. Energy requirement for lactating sow is high because they need energy to produce milk. Major constituent of milk is lactose and lactose is made up of glucose and galactose. Lactating pigs lose a significant amount of glucose in the form of lactose while they are producing milk. Gestating pigs are given restricted feed with less energy while lactating sows are allowed to feed ad libitum with the diet that contains higher amount of energy.

1.2.2 Protein requirement

Protein is considered as building block of body, while amino acids are building block of protein. Some amino acids are called essential amino acids as they should be supplied from feed, while others are called non-essential as they are synthesized within the body. Swine have specific amino acid requirement, not for crude protein. In past, crude protein used to be the guide which used to indicate the amount of protein required by pigs and was supposed that the feed ingredients supplied would provide the adequate amino acids requirement of swine. However, it is very important to weigh the amount of amino acids included in the diet of swine. It is generally considered a good practice to check the amount of amino acids, even when synthetic amino acids are provided along with agricultural co-products. Also, anti-nutritional factors present in byproduct can reduce the nutrient availability to pigs (Li et al., 1990). Protein from plant source has major effect on early weaned piglets, as their gastrointestinal tract is relatively less developed, so the proteins are not well hydrolyzed and their absorption is decreased (McDonald et al., 2001). Lysine is the first limiting amino acid in swine, that means if insufficient amount of lysine is included in the diet of pig, then protein synthesis in the animal

will not go further beyond the point to which lysine was made available. Thus, balancing lysine is of vital importance in pig diets. Requirement of lysine varies with age and stage of production. While formulating diets, lysine requirement is calculated first, then other individual amino acids are calculated based on the lysine ratio to make a protein balanced diet, which is also called as “ideal amino acid ratio”. Insufficient amount of any essential amino acid would affect the feed conversion ratio, consequently resulting in the depressed growth of animal. The most significant factor which affects the economy of any pig industry is the feed conversion ratio, as feed cost account for almost 60-70% of total production cost involved in pig industry. The younger the pig, the higher is the protein requirement; still newly weaned pigs are offered diet with less protein and with less amino acid than is actually required by some industry people, so that occurrence of diarrhea can be prevented. This low protein in the diet can lead to reduced gain, poor feed conversion ratio (FCR) and these pigs might end up with deficiency of one or more amino acid. On the other hand, finishers are meant to be grown faster with efficient FCR, thereby higher lean meat percentage can be expected.

Table 4. Amino acid requirement of pigs (NRC, 2012)

Amino Acid	11 lb pig(4.4 lb DMI/day) gm/day	440kg Gestating Sow(4.4lb DMI/day) gm/day	440 kg lactating sow (4.4lb DMI/day) gm/day
Threonine	1.83	2.37	3.02
Valine	1.58	2.01	3.5
Methionine	0.37	0.49	0.54
Isoleucine	1.17	1.58	2.06
Leucine	1.67	1.92	2.84
Phenylalanine	1.21	1.33	1.9
Lysine	1.34	1.85	2.64
Histidine	0.47	0.64	1.05
Arginine	0.73	0.91	2.04
Tryptophan	0.51	0.58	0.74

1.3 Agricultural products

1.3.1 Sweet potato

Sweet potato (*Ipomoea batata*) are a starchy tuberous plant belonging to family *Convolvulaceae*. Though only yellow flesh cultivars are well known to the consumers, there are different cultivars with varying flesh color. Adaptability to various environmental condition and high yielding capacity has attracted nutritionists towards the use of sweet potato both for human (Bouwkamp, 1985) and livestock nutrition (Dominguez, 1992). Sweet potato is a good source of energy with very high digestibility (around 90%) and a high biomass yield (An et al., 2003).

Sweet potato production is highest in Asia and the Pacific islands. China accounts for 80% of global sweet potato production while production of it is quite low in USA; only 0.8% of the global sweet potato is produced in USA (Truong et al., 2011).

Table 5. Sweet potato production in Hawaii, acres harvested, production and economic value (USDA, 2012)

Year	Acres harvested	Production, '000 lb	Economic value, '000 USD	Farm price, cents/lb
2007	420	7,100	3,621	51
2008	470	8,100	4,780	59
2009	600	8,300	5,413	65
2010	1100	12,600	6,510	52
2011	1100	16,700	7,348	44

Production of sweet potato has been increasing in Hawaii at a great pace. Sweet potato harvested increased from 420 acres in 2007 to 1100 acres in 2011. Area of farm allocated for sweet potato production almost tripled within the last 4 years. There is a significant increase in the production too. Sweet potato production increased from 7.1 million lb since 2007 to 16.7 million pounds in 2011. This significant increase in production is due to significant increase in land for sweet potato cultivation. Since the production of sweet potato has gone up, the price has been reduced. It used to cost 51 cents/lb during the year 2007 and hiked up to 65 cents/lb but then decreased to 44 cent/lb. Graves et al. (2010) reported that the introduction of sweet potato increased the agricultural potential of Big island of Hawaii by three times while it was increased

by two times in Maui island of Hawaii. Kurashima and Kirch (2011) reported that sweet potato cultivation has been initiated in colluvial soils, which has increased the agricultural potential of Molokai island by twice as much as before.

Nutritive value of sweet potato differs among the cultivars. This variation necessitates the nutritional evaluation of different cultivars of sweet potato before they are incorporated in the diet of swine. Purple sweet potato and okinawan sweet potato are two cultivars being considered in this study. Raffinose, stachyose and verbascose are some of the limiting factors present in sweet potato, found to cause flatulence in animals.

1.3.1.1 Purple sweet potato

Purple sweet potato is one of the varieties of sweet potatoes whose outermost skin is smooth and purple in color; and its interior flesh is also purple in color, giving its name.

1.3.1.2 Okinawa sweet potato

Outermost skin of OSP is white in color, whereas the interior is purple in color. This purple flesh of OSP is due to the presence of pigment called anthocyanin which acts as an antioxidant (Giusti and Wrolstad, 2003; Suda et al., 2003; Kano et al., 2005).

1.3.2 Taro

Taro (*Colocasia esculenta*) belongs to *Araceae* family (aroids). Taro, is a perennial plant that produces a starchy, tuberous root. It is a stem-less plant having heart-shaped leaves. Petioles are often purplish in color and are thick and succulent. The outer part of starchy tuber or the corm has rough ridges and spindly roots. Time of harvesting Taro is indicated by decrease in plant

height and yellowing of leaves. These signs are less distinct in taro that are grown in inundated areas.

Table 6. Number of farms, acreage and economic value of taro (USDA, 2013)

Year	Acres harvested	Production, '000 lb	Economic Value, '000 USD
2007	380	4,000,	2,360
2008	390	4,300	2,666
2009	445	4,000	2,440
2010	475	3,900	2,516
2011	485	4,100	2,747
2012	400	3,400	2,278

Taro plants are rich in starch. They are grown in all tropical and subtropical regions of the world, and is part of farming system in Hawaii. Taro is not only cultivated for its starchy corm but also for its heart-shaped leaf that is used as a vegetable. There are more than 200 cultivars of taro and all these cultivars are mainly classified into two groups. Upland taro produces eddos, which is then used like potato, while wetland taro's main corm is used to make poi. Taro can be grown easily whether the land is wet or dry, provided they get water either from irrigation or rainfall. Since wetland areas within Hawaii are gradually decreasing, taro production these days is more focused under dry land condition using irrigation. Though protein content in most of the tubers is relatively low, the protein content in taro is higher than in other tubers

(Onwueme, 1978). Furthermore, taro is a good source of some minerals like potassium, calcium and iron (John et al., 2007).

Taro contains calcium oxalate which causes itching of mouth and throat (Sakai 1979); a gelatin-like substance present in a cigar shaped capsule ejects raphids, resulting in itching. This can be controlled by cooking (Noonan and Savage, 1999; Enechi and Odonwodu, 2003), and drying also helps to a considerable extent. Fassett (1973) observed low-plasma calcium level and renal damage when high dose of oxalic acid was consumed. Other anti-nutritional factors present in taro are proteinase inhibitors, phytates, and tannins.

Taro production in Hawaii remained constant (around 4 million lbs) from 2007-2011, but there was considerable increase in the acres of land cultivated for taro production (from 380 acres in 2007 to 485 acres in 2011). In 2011, Hawaii earned around 2.7 million USD from the sale of taro. Production of taro drastically decreased from 4.1 million lb in 2011 to 3.4 million lb in 2012. Total production of taro decreased by 17%, or 0.7 million lbs, which in turn resulted in a total loss of about 0.47 million USD. This decrease in production was as a result of torrential rain in March 2012 which washed away entire crops of taro. In addition to this, later in the same year, a drought occurred, which affected the overall production (USDA, 2013).

1.3.3 Cassava

Cassava (*Manihot esculenta*) is a starchy, tuberous plant mainly used for human consumption as it ranks fifth among the staple crops consumed by 800 million people (Lebot, 2009), and also for feed for animals. Roots of cassava are low in fat and protein but rich in carbohydrate, and the starch granules in cassava is composed of 20% amylose and 80% amylopectin. According to FAO (2013), 256 million tons of cassava were produced globally in the year 2012, and there was

drastic increase in the production of cassava by 40% since 2000. The major production house of cassava is Africa, which contributes about 50% to the world's total cassava production.

The major limitation in the use of cassava as a primary source of animal feed is cyanogenic glycosides, linamarin and lotaustralin in a ratio of 97:7 which is present in the entire plant parts except seeds; however, the concentration is high in the leaves (Teles, 1995). Interior flesh of cassava has the least concentration of cyanogenic glycoside, while the peel contains the most. Cellular rupture causes hydrolysis of linamarin (cyanogenic glycoside) to glucose, acetone and hydrogen cyanide (Conn, 1994). Peeling, boiling, steaming, shredding, roasting, fermenting etc. are some of the methods that may be used to reduce the toxicity in cassava (Garcia and Dale, 1999). Cassava cultivation is easy as it is tolerant to acidic soils and can be grown in soils with low fertility. It is resistant to drought, and performs well even when the supply of water is limited and can resist adverse environment (El-Sharkawy, 2003). There are two varieties of cassava: sweet and bitter. The sweet variety of cassava produces greater yield, and so farmers more commonly grow it. However, to protect the crop from insects, farmers in South Africa prefer growing bitter varieties. Tivana et al. (2007) reported that farmers in South Africa have been practicing heap fermentation to reduce the bitterness in the root, and microbial growth occurring during the fermentation process results in softening of cassava roots reducing the cyanogens. Subsequent drying after fermentation would help in volatilization of remaining residual cyanogens. High moisture content in cassava limits its use in suckling and weaned pigs. The best way to feed cassava to the pigs is either as dried meal or in parboiled form.

1.4 Agricultural co-products

Conventional feedstuffs like corn and soybean are getting more scarce and expensive, hence agricultural byproducts/ mill byproducts can serve as potential alternative feed source to the livestock (Ensminger et al., 1990). Byproduct is referred to those residues either from plants or animals which is obtained during or after the production or processing for human food or animal feed (Fadel, 1999), and such byproducts may have the capacity to be utilized as feed for livestock.

The optimum utilization of these byproducts will be possible only if their nutritional content, digestibility and other potential limitation are identified; however, because of lack of this technical information, such byproducts are rejected as waste or are underutilized. These Agricultural co-products are produced in considerable amount almost everywhere in the world. Some byproducts might have the potential to be used in swine diet and some might not, but no recommendation can be made unless nutritional quality of these products is explored and evaluated. If these byproducts show potential and if they could be utilized in feed of swine or other animals, not only will they provide economic gain but will also help in preventing environmental pollution, by means of waste reduction. Farmers will also get benefited as they will be able to obtain feed of reasonable quality and less expensive rates for their animals than they would by buying traditional grains. Moreover, the feed industry also saves money that they might have to spend if they had to manage the waste. Attention of farmers, livestock producers, feed industries and nutritionists is focused more towards such byproducts these days. Despite containing anti nutritional factors, some of these byproducts are being used by feed industries. Multiple studies have been carried out over the past years regarding the use of some non-conventional feedstuffs in the pigs diet, and some of them have even showed promising results as a viable alternates like distiller's dried grains with soluble (Jha et al., 2010), tropical fruits,

leaves and tubers (Leterme, 2006), oil seed cakes (Seneviratne et al., 2010) and wheat millrun (Nortey et al., 2007; Jha et al., 2012a,b).

1.4.1 Macadamia nut cake

1.4.1.1 Macadamia nut and its production in Hawaii

Though Macadamia nut was discovered in 1857 by an Australian botanist (Ferdinand von Mueller, the Director of the Royal Botanical Gardens in Melbourne) and domesticated for the first time in 1858 in Australia, but it actually became well-known only after 100 years when it was successfully cultivated in Hawaii. It was named ‘Macadamia’ after the name of his good friend, Dr. John Macadam. Keauhou was the place in Hawaii where the first large-scale planting (truly commercial orchard) was started by establishing around 7,000 trees by Castle and Cooke in 1948. Although ten species have been identified, mainly two species are found to produce edible nuts which are grown commercially in Hawaii, Australia and New Zealand, mainly the rough-shelled ones. These are classified according to the structure of shell they possess, namely *Macadamia intergrifolia* having smooth shells while *M. tetraphyllais* the rough-shelled one. Macadamia is a tree having long life (productive life of 60 years or more), which starts to produce in six to seven years under favorable conditions (Rosengarten, 1984). The first processing factory was established in 1931. Research on cultivation of macadamias was undertaken by the Hawaii Agricultural Experiment Station.

Hawaii is one of the largest producers of the macadamia nut in the world. In the year 2013, 41 million lbs of worth 35 million USD was produced in Hawaii. Within the US, Hawaii is the only state where Macadamia nuts are grown.

Table 7. Macadamia nut production in Hawaii (USDA, 2014)

Year	Economic Value,	Production,	
	'000 USD	'000 lb	Acres bearing
2009	29,400	42,000	15,000
2010	30,000	40,000	15,000
2011	38,220	49,000	15,000
2012	35,200	44,000	15,000
2013	35,670	41,000	16,000

Production of macadamia nut is almost constant over the last five years, though the production was highest in 2011. Land involved in cultivating macadamia nut is increased by thousand acres if compared from 2012 to 2013. Kaijser et al. (2000) observed 30% moisture in the macadamia nut when harvested fresh, but the moisture content is reduced to 2% by drying so that they could be stored. Moisture content in the fresh macadamia nut was found to be 20.3% in the nuts harvested in year 2013 in Hawaii (USDA, 2014). Although the production decreased by 3 million pounds in 2013 compared to previous year, the gross income increased by 1%, due to rise in the price of nuts, which increased from 80 cents per pound in 2012-2013 to 87 cents per pound in 2013-2014.

1.4.1.2 Macadamia nut cake

Macadamia nut cake is derived from the oil extraction of Macadamia nuts. First, the nuts are pressed and oil is removed after centrifugation, and then the leftover product is considered as macadamia nut cake. It is considered as waste, hence it can be an inexpensive feedstuff produced

by the oil industry, since the cost attached to it is very minimal. It can be used by people living in rural areas to rear swine in small-scale production systems, since it is the cheapest byproduct that can be obtained for relatively no cost. The nutrient content of MNC varies with the quality of the macadamia nut, the source from where it is retrieved, exposure to rain, climatic condition of the place where it is grown and the processing technique that has been applied. Processing techniques applied, i.e. the extrusion process adopted by the oil producing industry, also affects the nutritive value of MNC to a large extent. Hence, chemical composition from every single batch should be evaluated before being used to formulate any diet for swine or other livestock. The limiting amino acid present in MNC is threonine. Highly varying concentration of fiber content in MNC is the biggest limitation of MNC as feedstuff, and might make it unsuitable for inclusion in the diet of swine or any other monogastric animals if diets are not formulated properly. Crude fiber content in MNC is very high whereas the available energy is relatively low; hence, it is called as “intermediate product” (Van der Merwe and Smith, 1977). There is very limited published information about MNC with no information available on its use in the swine diets.

1.4.2 Wheat mill run

Wheat millrun is a powdery particle which is obtained from a wheat milling industry, i.e. when flour is extracted from wheat or durum (a hard wheat variety) during milling, the byproduct left is called wheat millrun. Wheat millrun is also referred to as wheat middlings or wheat midds in general, but wheat middlings contain a lower proportion of wheat bran (coarse outer covering of wheat kernel), while 28% of intact kernel is found in wheat millrun. Nutritive value of wheat millrun varies to a considerable extent, depending on the milling industry, quality of wheat and the process by which wheat millrun is extracted. There are different varieties of flour that are extracted

from wheat; quality of flour desired significantly affects the quality of wheat millrun. Yield decreases as the purity of the flour increases. This variability might be one of the limits to use of WMR in the pigs diet. Nortey et al. (2007) found 7% decrease in average daily gain when 40% of wheat millrun was included in the diet of growing pigs. However, growth performance was not affected when 30% of wheat millrun was included in the diet of finisher pigs (Stewart et al., 2013).

1.4.3 Barley brewers grain

Fermentation of malt obtained from digestion of barley results in the production of beer, but the byproduct obtained in the beer-making process involving barley is called barley brewers grain. This malt is produced by soaking barley, allowing it to germinate and then drying it. Milling and subsequent soaking of the malted barley in hot water causes transformation of starch into sugar, by action of enzymes. This sugary liquid produce is boiled, filtered and fermented to produce beer. Once all the sugar from the grain has been removed the resulting product is called barley brewers grain. The used grain is dried out during the recycling process, which makes it lighter. This used grain after brewing is considered as waste product as it is of no use to the brewery. As only starch is primarily used during fermentation, other nutrients like protein, fiber and others still remain in the waste, even at higher concentration than the parent grain. The nutritive value of brewers grain varies with the process used to make beer. First limiting amino acid in pigs is lysine, but this brewers grain consists of very low quality protein, and high amount of fiber which can be a limiting factor in use of this waste in pig diets. As it is easy to store and is more stable, the dried grains are fed to pigs (Blair, 2007). Feed intake capacity is lower in younger pigs, so they eat less and require nutrient-dense food while sows in gestation period require relatively low amount of energy, and still are capable of eating more than what they actually

need. So, Holden and Zimmerman (1991) recommended not using Barley brewers grain in starter diet, but that it can be used in substantial portion in the gestating pigs diet, and at a lower inclusion rate in lactating, finisher or grower animals diets. However, Young and Ingram (1968) did not find any significant reduction in weight gain, when 23% of brewers grain was included in the growing pigs diet. Young and Ingram (1968) successfully used dried brewers grain to supply 50% of the supplemental protein without affecting the animal's performance.

1.4.4 Okara

Tofu-making process results in a production of soybean solid byproducts referred to as okara. All of the milk in okara is squeezed out. It is white or yellowish in color. Okara is a kind of soy fiber. Every kilogram of soybean used in the tofu-making process results in about 2.64lb production of Okara (Li et al., 2008). Although it is rich in protein and dietary fiber, it has a short shelf-life due to its high moisture content (around 80%). It is low in demand as human food. Thus, it can be made available for animal feeding program. Leaving okara untreated during the summer causes aerobic degradation within few hours. Once the degradation process is underway, its palatability is reduced and may cause diarrhea when fed to animals. This putrefaction is because of high water activity in okara. Anti-nutritional factors present in okara are trypsin inhibitors, saponin and lectin. Amount of water phase extracted from soybeans highly affects the chemical composition of okara. Redondo et al. (2008) reported lipid content in dried okara as 10% and crude protein as 25%.

1.5 Anti-nutritional factors

The anti-nutritional factors are compounds in feedstuffs which affect the health of animal, retard the growth either by themselves or through their metabolite by interfering with the utilization of

available nutrients present in the diet (Huisman and Tolman, 1992). Common ANFs found in the studied feedstuffs are presented below.

1.5.1 Cyanogenic glycosides

Cyanogenic glycosides are water-soluble compounds that are formed in the cytoplasm but stored in vacuole of plant cell; the enzyme that degrades these glycosides occurs in the cell wall.

Cyanide toxicity occurs only when these cyanogenic glycosides come in contact with the enzymes during grazing, intense heat, frost etc. The hydrolytic process, which takes place when these enzymes come in contact with cyanogenic glycosides, results in the production of three components: hydrocyanic acid (HCN), a sugar molecule and a ketone or an aldehyde group, of which the HCN is poisonous. These cyanogenic glycosides in plants are non-toxic until HCN is released during the hydrolytic process or by rumen microorganism in ruminants. Linamarin and lotaustralin are the cyanogenic glycosides present in cassava. These cyanogenic glycosides are not produced in roots, but in leaves, and are then translocated to root system via phloem (Jorgensen et al., 2005). Cyanogenic glycoside is present in all parts of plant (in cassava) except in seed; however, the concentration of linamarin is low in tuberous roots and highest in the peel. Concentration of HCN varies between parts of the plant. Cyanogenic glycosides are normally derived from amino acid. The two cyanogenic glycosides present in cassava are linamarin (93%) and lotaustralin (7%), and are derived from the amino acids: valine and isoleucine, respectively. Linamarase and linase are the endogenous enzymes present within the cell wall of cassava (Mkpong et al., 1990) which is responsible for hydrolysis of these glycosides producing β -D-glucopyranose and 2-hydroxyisobutyronitrile or acetone cyanohydrins which later get dissociated to toxic HCN and acetone by hydroxy nitrile lyase. If the dissociation of these glycosides occurs in presence of concentrated acid or concentrated base, then the end product is

not HCN, which makes it non-poisonous. Non-ruminants like pigs are less sensitive to these glycosides, as they have acidic stomachs, while ruminants are more susceptible to it. Lethality of cyanide poisoning is associated with inhibition of respiration, which occurs from the inhibition of cytochrome c-oxidase; a carrier protein in the electron-transport chain. Cells in the central nervous system and heart are more affected as they depend on aerobic respiration, these cells will not be able to produce ATP through aerobic mechanism once the electron transport chain is disrupted, as they are not able to extract oxygen from the blood.

Sodium nitrite and sodium thiosulphate are used in combination when cyanide poisoning occurs. Sodium nitrite causes formation of methamoglobin by converting ferrous ion in the haemoglobin to ferric form. As affinity of cyanide is more towards methamoglobin, they leave cytochrome c-oxidase and are drawn towards methamoglobin forming cyanomethamoglobin. Sodium thiosulphate acts with cyanomethamoglobin forming thiocyanate in the presence of hepatic enzyme rhodanase. This thiocyanate is excreted through the kidney.

Herbicides, frost and drought can increase HCN concentration, while drying plants and ensiling them can reduce toxicity by liberating HCN during curing.

1.5.2 Phytic acids

Phosphorus in many plant tissues is stored in the form of phytic acid; a cyclic compound also present in macadamia nut cake. Most of the grains contain phytic acid in the aleurone layer, while in legumes it is present in the cotyledon layer to an extent of 1-5% of the dry weight. It is considered ANF as it not only reduces the phosphorus availability but also acts as chelating agent and chelates calcium, magnesium, iron and zinc, thus inhibits absorption and bioavailability of these minerals (Reddy et al., 1982; Pallauf and Rimbach, 1997). Digestive

enzyme phytase is required to release phosphorus from phytate molecule, but non-ruminants like pigs do not have this enzyme. Hence, neither they can extract phosphorus, nor they can utilize other minerals in the presence of phytic acid. Instead, the requirement for minerals in such animals is increased (Cromwell, 1992). Impact of phytate has also been seen on carbohydrate and protein digestibility, as it has been found to form complex with basic residues of proteins, then inhibits digestive enzymes such as pepsin, pancreatin and amylase (Gifford and Clydesdale, 1990).

Heat treatment not only denatures phytate but also the enzyme phytase. Absorption of phosphorus in growing pigs was found to increase when diet was soaked in whey for 3 hours at 40°C (Nasi et al., 1995). Adding exogenous microbial phytase enzyme in pig diets has been found to improve phosphorus digestibility (Helander et al., 1996; Poulsen et al., 1999).

1.5.3 Oxalates

Oxalate is one of the anti-nutritional factors present in plants like taro, which is actually a salt derived from oxalic acid. These oxalates form a strong bond with various minerals like sodium, potassium, calcium, magnesium etc. Though the oxalate salt of sodium and potassium are soluble, oxalate salts of calcium are generally insoluble. This insoluble salt solidifies within the renal and urinary tract forming kidney stones or urinary calculi (Blood and Radostits, 1989), blocking the renal tubules. Oxalates interfere with the metabolism of nutrients, reduces their bioavailability, cause various nutritional deficiencies, and also irritate the lining of gut. However, oxalates are not much of a problem in ruminants, since both the soluble and insoluble oxalates can be metabolized in the rumen by ruminal microbes. Cooking helps to reduce the toxic effect of oxalates (Enechi and Odonwodu, 2003).

1.5.4 Tannins

Hydrolyzable tannins and condensed tannins (which do not hydrolyze) are the two groups into which tannins are classified. Tannins create a major impact on the digestibility of protein by inhibiting protein-digesting enzymes such as trypsin, chymotrypsin, amylase and lipase. Effects of tannin in monogastric animals like pigs are seen in different ways. These affect animal performance either by binding to minerals and other nutrients and making it unavailable for digestion, or by affecting digestive enzymes, intestinal irritation and digestive disorder (Makkar et al., 1987). In ruminants, it has been found to affect digestibility by reducing the feed intake (McLeod, 1974). Pigs and birds are unable to completely eliminate effects of tannins.

1.6 Determining digestibility of feedstuffs in swine

It is very important to get the proper prediction and estimates of nutrient composition and digestibility of every feedstuff so that feedstuffs can be effectively used in the animal's diets. Knowledge about digestibility of feedstuffs helps animal nutritionists to formulate a diet as required by the animals. As the diet is formulated based on the nutrient composition and digestibility, it helps to prevent or minimize food from being wasted or overfed. If the knowledge about these feedstuffs is not proper and predicted value of the feedstuff that are used to formulate feed is not accurate then this might result in variable and often poor performance of animal than what was actually anticipated to be. Feed that is formulated based on the improper knowledge about the feedstuff can result in incorrect inclusion of the feed ingredients that might meet or might not meet the nutrient requirement of animal. Some consequences of such incorrect nutrient formulations may be increased feed cost and poor performance of animals.

Energy and protein are major components of any diet. The single factor that has the potential to affect the cost of animal production is the feed, which provides source of energy to the animals. Hence, determination of apparent total tract digestibility of any feedstuff and estimation of digestible energy in the feedstuff (which provides actual source of energy on routine basis) is very important before they are used in any swine diet. This is because the same feed ingredients may have variable nutrient composition in different batches, therefore the feeding value might change due to processing. To evaluate digestibility of feedstuffs, different techniques, as listed below, have been developed and are being used:

- In vivo methods
- In vitro methods
- Near infrared reflectance spectroscopy

1.6.1 In vivo method

Feeding trial or in vivo study is the best method that has been used to estimate digestibility of feedstuffs. This method may not be suitable for routine feed evaluation from practical point of view as animal trials are expensive, time consuming and laborious which limit use of in vivo method on routine basis. The number of animals required in animal trials varies depending upon the experiment that is being conducted; still, the number associated with it is naturally high (Ellis and Hill, 2005). To manage animals in a small confined area can also be problem. Besides this, there are a lot of logistical limitations associated with any experimental design to conduct in vivo studies. Some digestibility studies use in vivo method as a gold standard to compare the result observed using different methods. Any other alternative methods can be critically evaluated or validated only if there is availability of accurate data generated from the in vivo studies (Coles et

al., 2005). According to Schneider and Flatt (1975), any digestibility study that uses animal subjects is conducted over three phases: the first phase is termed as transition phase where animal is allowed to feed over a diet, second phase includes acclimatization phase where animal is allowed to get acclimatized with the test diet. Duration of acclimatization phase depends upon the variation between the physical and chemical composition of diet used in the transition phase and acclimatization phase. If the variation is large, it is usually better to increase the duration of acclimatization phase, so that the animal will have sufficient time to get adjusted to the new diet and also if the animal is placed within a new environment. The third phase is the collection phase where samples required are collected and evaluated depending upon the desire of the study. To get digestibility, either whole fecal collection or marker method are used; marker method is preferred and commonly used. Markers are chemicals which are not digested at all in the animal body. In vivo studies are highly vulnerable to the errors associated with the use of these markers, also with the different kinds of variations associated with the use of different species of animals used in the study.

If the study is conducted to get the idea about the ileal digestibility, either the animal has to be surgically operated to fistulate or killed at the end of the study to collect the ileal sample. Fistulation and other processes require surgery, which in turn, requires technical know-how; such technically expert personnel may act as limitations for the use of in vivo studies. Post-operative infections are also associated with surgery which might affect the results of the experiment.

1.6.2 In vitro method

Predicting behavior and digestibility of feedstuff that occurs in real body of the animal or when used in in vivo method has been successfully adopted in humans, swine, poultry and other monogastric animals. These predictive processes have evolved after series of studies conducted in past over several years beginning with the work that was originally conducted in ruminant animals in the year 1963 by Tilley and Terry. Both neurons and hormones control the digestion and absorption of feedstuff taking place in live animals. No artificial setup can be as accurate as the one taking place in the body of animal, or include all the physiological events taking place in the body of animal (Fuller, 1991) and there are lot of challenges associated with this (Moughan,1999). Microbes and microbial environment that occurs in the large intestine is very complex. Certainly, in vivo method is the best method to get the accurate digestibility of feedstuff. However, in vivo is an expensive, time consuming and laborious method. There are a lot of logistical limitations associated with this method. In vivo method is also subject to error associated with the technical skills and expert manpower. Most importantly, it is not suitable for evaluating digestibility of single feedstuffs as no single feedstuff can fulfill all the nutrient requirements of animal. Various kinds of markers such as digesta flow rate markers, microbial markers etc. are used in in vivo method to get the digestibility, but different kinds of errors are associated with the use of these markers as well as errors associated with variation among the animals (Stern et al., 1997). Therefore, in vitro method is the preferred means for evaluating digestibility of feedstuffs, as this method can be used to screen a large amount of sample within a short period of time and with relatively lower cost involved. Near-infrared reflectance spectroscopy (NIR) is equipment which is used to evaluate the nutrient quality of feed very rapidly; however, this instrument can function well only when it is calibrated properly. Most

importantly, animals are not involved in this process, thus animal welfare is not compromised. In vitro method can be critically evaluated or validated only if there is availability of accurate data analyzed with in vivo method.

Enzymatic digestibility assays (Boisen and Fernandez, 1997; Noblet and Jaguelin-Peyraud, 2007) have been used with greater accuracy to predict apparent total tract digestibility of feedstuffs as well as compound feed. Several workers (Chen,1997;Spanghero and Volpelli,1999) confirmed that the process occurring in the gastrointestinal tract of pigs can be simulated effectively by in vitro method as proposed by Boisen and Fernandez (1997). Instead of using microorganism which is responsible for fermentation, they used purified enzymes which are available commercially. Boisen and Fernandez (1997) used a cocktail of fiber-degrading multi-enzyme complex, commercially available as Viscozyme (Novozymes, Bagsvaerd, Denmark) to digest fiber; a process taking place in the hindgut of pigs whereas Huang et al. (2003) only used cellulose as a fiber-digesting enzyme to mimic the hindgut. No eliminating process such as absorption takes place in in vitro method, since it only deals with enzymatic solubilization. Fecal inoculums containing active microbes have been used to mimic activity taking place in the hindgut (Jha et al., 2011a,b).

The three-step process in vitro digestibility methods developed by Boisen and Fernandez (1997) simulates similar kind of condition occurring in the gastro intestinal (GI) tract of pigs, all the way from pH, temperature, time of passage and various enzymatic action taking place in GI tract of pigs. The first step attempts to simulate the stomach where pH is maintained at 2 using hydrochloric acid, time of passage at two hours, temperature maintained at 39°C whereas pepsin as the enzyme released in the stomach. The second step tries to mimic various activities occurring in the small intestine where pH is maintained at 6.8 using sodium hydroxide, sample is

incubated at 39°C for four hours and enzymatic action was mimicked by using pancreatin. Combining these two methods gives a clear picture of the digestive process that takes place in the foregut which predicts the apparent ileal digestibility. The enzymes pepsin and pancreatin, used in first and second steps, are the commercially available enzymes which do not contain any microbial enzyme and are consistent in composition. Feedstuffs which are incubated in the water bath have to be stirred to prevent the feed particles from settling down at the bottom. While settling down, these feed samples may get poorly dispersed and may not be easily available for enzymatic action. Stirring is also necessary to mimic the peristaltic movement taking place in the gastro intestinal tract of pigs, which is done while keeping the samples in shaking water bath at 50rpm. The third step simulates the digestive process within the large intestine or hindgut. The feedstuffs are incubated at 39°C for 18 hours and pH is maintained at 4.8. Instead of using microbes, it uses multi-enzyme complex for fiber digestion. No microbial activity takes place in this step as microbes are not being used, hence there is no real fermentation occurring as purified enzymes are only capable of simulating the digestion but incapable to ferment the substrate. Only microbes and microbial enzymes produce gases as products and also yield energy through production of volatile fatty acids (VFA) by breaking down the substrates into simpler form. Proteins in the sample are solubilized by using sulfosalicylic acid at the end of this step. Feedstuffs are filtered using filtration funnel lined with filter paper (Whatman no. 54) to get the apparent total tract digestibility. Viscozyme contains different carbohydrates degrading enzymes such as cellulase, β -glucanase, arabinase, xylanase, mannanase, and pectinase. The major advantage of enzymatic method is that it does not require animal as inoculums donor. In other words, fecal sample need not be brought from pigs as the inoculums vary globally in different types of pigs due to being raised in different environment and feeding condition. Although

Holter (1991) describes that the microbes remain alive and are viable for several hours after they are excreted or collected, the quality of inoculums prepared deteriorates in proportion to the time spent between sample collections and using as inoculum in the feed samples (Vince et al., 1976). It is easy to transport enzymes; however, to transport fecal inoculums, accurate temperature and nutrition have to be maintained so that the microbes can survive until being used.

Limitation of *in vitro* methods

- The standard protocol available for enzyme preparation makes it advantageous over using microbes, as the former are more reproducible (Moughan, 1999). There is no necessity to search for inoculum donor pigs, when the animals have already been treated with antibiotics, since inoculum donors need to have a clear representation of microbial populations. However the enzymes that are selected to mimic hindgut might not be representative of all the microbial enzymes produced by all the microbial species residing in the hindgut of pigs.
- Enzyme: substrate ratio and the time these enzymes and substrate are allowed to interact with each other highly influence the accuracy of *in vitro* methods. Higher concentrations of pepsin and more time allowed for enzymatic action might result in complete digestion of even poor quality protein resulting in inaccurate results.
- Different types of dietary fiber affect the time of passage or the transit rate, and mimicking this effect of fiber in *in vitro* set up is difficult.
- Different kind of anti-nutritional factors are present in different kind of feedstuffs. This anti-nutritional factor exerts various kinds of effects on physiological and biological processes taking place in the body, which might also affect the digestion and absorption of nutrients. This effect cannot be addressed within *in vitro* systems.

➤ No eliminating process such as absorption takes place within in vitro method, as it only deals with enzymatic solubilization i.e., it assumes all the soluble materials as digestible material. Such soluble materials may contain proteins that might have been broken down into small peptides during the course of digestion but might not have absorbed in vivo. Solubility and hydrolysis are different from each other. Materials which are soluble does not mean they are completely hydrolyzed and converted to monomeric form which can then be absorbed when used in vivo.

Despite some limitations and disadvantages associated with the use of in vitro methods, and that it might not be 100% in alignment with the in vivo results, it cannot be said that this method is of no use in practical evaluation of feed. In fact this method is highly repeatable and can be best applied when large amount of samples are to be screened in short duration with limited resources. Thus, the in vitro methods are increasingly used in routine feed evaluation program now.

1.6.3 Near-infrared reflectance spectroscopy

A very rapid method of analyzing the nutrient quality or chemical composition of feedstuff is by using an instrument called near-infrared reflectance spectroscopy. Infrared wavelengths cannot be detected with the naked eye, as such wavelengths are longer than the visible range (400-700nm); however, the heat caused by it can be felt. The major principle on which this sophisticated analytical technique is based is that the major component of every feed sample has near-infrared absorption property. This spectral property of feedstuffs can be used to determine the chemical composition of every feed sample. The software searches the database for the sample spectra, and it looks for close relatives that resemble the sample being analyzed. In other words, organic matter is the major constituent of every feedstuff. The molecular bond between

hydrogen, nitrogen, oxygen, carbon, sulfur and phosphorus that exists in this feedstuff generally absorbs light either in or beyond the region of near-infrared. Hence this NIR equipment measures the difference between the light emitted by the equipment and the amount of light absorbed and/or reflected. To improve the accuracy and precision of this instrument, a lot of progress has been made over the past several years, not only in instrumentation but also in calibration, due to technical advances made in computer hardware and software. Though NIR was developed during 1950s, its actual use in the world of feed science or evaluating chemical composition of feed was applied in 1970s (Norris et al., 1976). This method has been approved by Association of Official Analytical chemists (AOAC) to determine crude protein and acid detergent fiber (AOAC 989.03) and for determination of moisture (AOAC 991.01). Wrigley (1999) used it to determine starch and non-starch polysaccharide (NSP) in feed grains and Murray (1993) used it in dried forages.

The major advantage of this method is that it is a less expensive in routine use, when compared to other methods. The cost involved in analyzing a single sample by wet chemistry will be less than or equal to the one obtained by running multiple sample using NIR. Furthermore, this method does not require any sample preparation and a minimal amount of sample is sufficient to analyze several parameters of the same sample within a short period of time. The most important aspect of NIR is that it is based on absorption and reflectance of light, and it does not use any chemical or reagents, hence no chemical waste will be generated while samples are analyzed using this technique.

The major limitation with the use of NIR is the calibration process, which takes considerable time. Also, a large amount of samples have to be used to calibrate the instrument. For example, if the instrument needs to be calibrated for sugarcane silage. In such conditions, these different sugarcane samples have to be analyzed, given that they are grown in different

climatic as well as geographical conditions. Therefore, in order to calibrate for a single feedstuff, hundreds and thousands of samples from that feedstuff have to be analyzed which gives the power to accurately predict the nutritional value of the samples. As calibration is required, this technology is applicable to only those feedstuffs for which calibration database has been developed. Also, it is an expensive instrument, thus initial investment is high, despite being lower running cost on routine basis.

1.7 Hypothesis of the study

The selected agricultural products and co-products have desirable nutritional profile and digestibility to be used as alternative feed ingredients in swine feeding program.

1.8 Justification of study

Traditional feed ingredients like wheat, corn, and soybean are typically in limited supply and expensive. Their production has never been sufficient enough to meet the demand of both human and livestock consumption. The higher cost and irregular or limited availability of these traditional feed ingredients have together paved a new path for the nutritionist to explore for alternative feedstuffs that can either completely or partially replace the inclusion of these traditional feed ingredients in the swine diets. Potential alternative feedstuffs are available and can fulfill the nutritional requirements of livestock (FAO, 1997) and reduce feed costs (Woyengo et al., 2014). Several alternative feedstuffs, including cassava (Gomez, 1991), roots, bananas, plantains, organic waste (FAO, 1997), tropical fruits, leaves and tubers (Leterme, 2006), taro (Buntha et al., 2008), novel grains and pulses (Beltranena and Zijlstra, 2007), distiller's dried grains with solubles (Jha et al., 2010), oilseed cakes (Seneviratne et al., 2010) and wheat millrun (Nortey et al., 2007; Jha et al., 2012a,b) have been evaluated and used successfully in swine diets

elsewhere in the world. However, agro-climatic conditions and farming system largely differ from location to location, which thereby influence the nutritional profile of feedstuffs and their utilization in animals.

In order to assure sustainability of swine production in areas like Hawaii and other Pacific islands, where traditional feed ingredients either cannot be grown or cannot be destined to animal feeding, alternative feeding system need to be studied, developed and extended. Some agricultural products might serve as a source of energy like purple sweet potato, okinawan sweet potato, cassava, taro are available locally in Hawaii. Similarly, some co-products which might serve as a source of protein like macadamia nut cake, okara, wheat millrun and barley brewersgrain are available locally. These agricultural products/agricultural co-products could together provide basis for producing more affordable locally manufactured feeds. However there is very limited or no information available on nutritional value and digestibility of these potential feedstuffs, which limits their use in routine swine feeding program. Thus, details information on the nutritional value of these potential local feedstuffs is warranted before considering them for sustainable swine nutrition planning in Hawaii. Moreover, disposal of these byproducts results in economic loss and potential environmental impacts in Hawaii each year (DOA Hawaii, 2007). Identification and information on nutrient utilization of potential local feedstuffs will not only improve the vigor of the swine industry through a reduction in feed cost, but also enhance the sustainability of environment by reducing waste disposal.

1.9 Objective of the study

The primary objective of this study was to determine the nutritional value (chemical composition) and in vitro digestibility of some of agricultural products and co-product either grown or are available locally in Hawaiian swine.

Chapter 2: Materials and Methods

2.1 Feedstuffs

Agricultural products including purple sweet potato (PSP), okinawan sweet potato (OSP), cassava, and taro were selected as a potential source of energy. Agricultural co-products like macadamia nut cake (MNC), wheat millrun (WMR), barley brewers grain (BBG) and okara were collected as a potential source of protein and fiber.

Agricultural products (PSP, OSP, Cassava, and Taro) were obtained from local farmer's market in Honolulu, HI and were of unknown origin during May 2013. Collected samples were ground and stored as required for different nutrient analyses and to determine in vitro digestibility of dry matter, gross energy and crude protein.

Macadamia nut cake was sourced from a local macadamia nut miller (Oils of Aloha, Kunia, HI). Other co-products (barley brewers grain, okara and wheat millrun) were sourced from a local pig farm (Wong's Pig farm, Waianae, HI).

2.2 Sample preparation

All the collected samples were chopped by using a knife and dried in a hot air oven to get the dry matter. The dried samples of agricultural products as well as agricultural co-products were further ground using Wiley mill (Thomas Model 2 Wiley® Mill – Thomas scientific) to pass through 1 mm screen to get a uniform particle size. The samples were also ground using 0.5mm screen for some nutritional analysis. Nutrient profile as well as in vitro digestibility of samples were determined using 1mm ground sample while starch was determined using 0.5mm ground sample. All the feedstuffs were coded with a specific number accordingly as required for

different analytical process; the same code was used throughout the study. The samples were stored in cool and dry place until further analysis.

2.3 Nutritional analysis

Proximate analysis of the samples was conducted according to the Association of Official Analytical Chemists standard procedures (AOAC, 2007). Other analyses were conducted using specific procedure, as mentioned in corresponding sections below.

2.3.1 Dry matter

Whatever is remained after removing moisture from a feedstuff is referred to as dry matter. Nutrient in a feed is measured from a dried sample obtained after removing the moisture portion. It is very important to know the amount of moisture present in a feed, as it does not provide any nutrient to the animals but does affect the weight of the feed.

The DM content of the samples was determined by AOAC method (930.15). For that, aluminum crucible was dehydrated by drying in an oven and subsequently cooled in desiccators. One gram of sample was taken in a weighed aluminum crucible. The sample was placed in hot air oven at 135°C for two hours. Whatever weight was lost was considered as loss of moisture. Dry matter was calculated using following formula:

$$\% \text{ of DM.} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where,

W1 = Weight of an empty crucible

W2 = Weight of crucible with sample before drying

W3 = Weight of crucible with sample after drying

2.3.2 Total ash

Total ash content of the samples was determined by AOAC method (942.05). One gram of dried sample was taken in a pre-weighed porcelain crucible after drying and cooling in a desiccators, so that moisture in the crucibles was removed. The sample was ashed in a muffle furnace at 600°C for four hours. The ash content was calculated as follows.

$$\% \text{ of Ash} = \frac{W2 - W1}{W} \times 100$$

Where,

W = wt. of dry sample taken

W1 = wt. of an empty crucible

W2 = wt. of crucible with ash

2.3.3 Crude protein

The nitrogen (N) content in feedstuffs was analyzed by dry combustion using a LECO CN-2000 analyzer (Leco Corp., St. Joseph, MI). The crude protein content of samples was determined by multiplying N content in feedstuff by 6.25 (AOAC990.03). Blank EDTA samples were run to correct for drift before starting the actual protein analysis. Then blanks and EDTA were calibrated and actual sample analysis began. 100mg of sample was weighed and placed on the sample wheel. After correcting blanks and the EDTA drift, the software with the LECO machine determines the protein value without any other inputs besides the sample weight. The CP content received is on as is basis.

2.3.4 Acid detergent fiber

Acid detergent fiber (ADF) refers to the non-solubilized residue that remains after boiling a feed sample in acid detergent solution. It contains cellulose, lignin silica and other nitrogen which are insoluble, but no proportion of hemicelluloses, i.e. it includes indigestible portion of feedstuff.

Higher the ADF content in a feed, lower is the energy digestibility. It provides rapid method for determination of lingo-cellulose in the feed sample.

ADF content in samples was determined following filter bag technique (AOAC 973.18) using Ankom²⁰⁰ fiber analyzer (Ankom Technology Corp., Macedon, NY). Acid detergent solution was made by adding 20gm of acetyl tri-methyl ammonium bromide (CTAB) to 1 liter of 1N H₂SO₄. Filter bag was dried in an oven for an hour and cooled in a desiccators to remove the moisture content in it. 0.5gm of samples were sealed in a pre-weighed filter bag and placed in the bag suspender. 2000ml of acid detergent solution was added to the digestion vessel which was agitated and heated for an hour. After the time has elapsed the exhaust pipe was opened to drain the solution. After that 2000ml of hot water (90-100°C) was added to the digestion vessel which was agitated for 3 minutes; the same process was repeated thrice. Filter bag was taken out and soaked in acetone for 3 minutes. All the filter bags were taken out, and was allowed to stand in room temperature for a while so that all the acetone gets evaporated, which can cause a flame when kept in oven. The samples were then dried in hot air oven for 4 hours at 105°C.

$$\% \text{ of ADF} = \frac{(W_3 - (W_1 \times C_1))}{W \times \text{DM}} \times 100$$

Where,

W3 = Weight after extraction process

W = Sample weight

W1 = Weight of filter bag

C1 = Blank bag correction (final oven dried weight / original blank bag weight)

2.3.5 Neutral detergent fiber

Neutral detergent fiber (NDF) refers to the non-solubilized residue that remains after boiling a feed sample in neutral detergent solution. It contains cellulose, lignin as in ADF, and also

contains hemicelluloses (Pond et al., 2005). Glucose molecule in cellulose is linked by β (1-4) bonds instead of α (1-4) and α (1-6) as in starch. Hemicellulose is a term used for series of polysaccharides like xylose, arabinose, mannose etc, which can be extracted with alkali. Pectin is composed of galactose, arabinose, rhamnase etc; in general pectin is present in cell wall of plant having complex structure but is not expressed in NDF as they are soluble in neutral detergent solution. Lignin is a complex structure formed by cross linking of phenolic units.

Heat stable α -amylase and sodium sulfite was used to determine NDF in an ANKOM²⁰⁰ fiber analyzer (Ankom Technology Corp., Macedon, NY). Neutral detergent solution was made by mixing 1199.6gm of neutral detergent concentrate (ANKOM Technology, Macedon NY) with 200ml of tri-ethylene glycol and double distilled water was added to make up to 20 liters. F57 Filter bag (ANKOM Technology, Macedon NY) was dried in an oven for an hour and cooled in a desiccators to remove the moisture content in it. 0.5gm of sample was sealed in a pre-weighed filter bag and placed in the bag suspender. 2000ml of neutral detergent solution was added with 20gm of sodium sulphite and 4ml of heat stable α -amylase to the digestion vessel which was agitated and heated for an hour. One hour later the exhaust pipe was opened to drain the solution. 2000ml of hot water (90-100°C) with 4ml of α -amylase was added to digestion vessel and agitate for 3 minutes; the same process was repeated thrice. Filter bag was taken out and soaked in acetone for 3 minutes. All the filter bags were taken out, and was allowed to stand in room temperature for a while so that all the acetone gets evaporated, which can cause a flame when kept in oven. The samples were then dried in hot air oven for 4 hours at 105°C.

$$\% \text{ of NDF} = \frac{(W_3 - (W_1 \times C_1))}{W \times \text{DM}} \times 100$$

Where,

W3 = Weight after extraction process

W = Sample weight

W1 = weight of filter bag

C1 = Blank bag correction (final oven dried weight / original blank bag weight)

2.3.6 Gross energy

Gross energy of both agricultural products and Agricultural co-products was determined by using oxygen bomb calorimeter (Parr 6200, Parr Instrument Company, Moline, IL) and expressed as Kcal/kg. Samples were weighed 1gm and placed in the crucible. Crucible was placed in 1108p oxygen combustion bomb (Parr 6200, Parr Instrument Company, Moline, Illinois) filled with oxygen. The samples were ignited in sealed decomposition vessel (Oxygen combustion bomb), in oxygen rich environment. Benzoic acid was used to calibrate the heat capacity of calorimeter system; increase in temperature of water in the bucket within the inner vessel was measured automatically. The software linked with the instrument calculates the energy value automatically.

The nutrients concentration received after the analysis were on “as is basis” and were converted to “DM basis” using following formula:

$$\% \text{ of nutrient (DM basis)} = \frac{\text{Nutrient \%}}{\text{Dry matter \%}} \times 100 \%$$

2.3.7 Starch

Starch content of samples was determined using test kit (Megazyme International, Ireland; AOAC 996.11), maize starch was used as reference sample. Briefly, 0.1gm sample was weighed in glass test tubes (16×120 mm). Sodium acetate buffer (200mM, pH4.5) was kept in pre-warmed water bath at 50°C. 0.2ml of 80% ethanol was added to tube and vortexed. Immediately 2ml of dimethyl sulphoxide was added and vortexed and the tubes were placed in hot plate at

100°C for 5 minutes. Three ml of MOPS containing α -amylase (1ml α -amylase in 30ml of 50mM MOPS buffer (pH 7)) was added to the test tubes and kept in dry block for 6 minutes, vortexing after every 2 minutes. Tubes were then placed in hot water bath at 50°C. Four ml of pre-warmed sodium acetate buffer along with 0.1ml of amyloglucosidase was added to the tubes, mixed gently and covered with parafilm. The samples were incubated at 50°C for 30 minutes in water bath. The volume was adjusted by mixing 0.7ml of distilled water and the samples were centrifuged @3000 rpm for 10 minutes. About 1ml of supernatant was transferred to a new tube and diluted to 10ml by adding 9ml of distilled water. Two blank was prepared by adding 0.1ml of distilled water, two glucose standard and 0.1ml of aliquote of each sample was transferred to a tube. To the tubes, 3ml of GOPOD reagent was added and covered with parafilm and incubated at 50°C for 20 minutes in a water bath. The absorbance was read at 510nm using the blank to zero the spectrophotometer.

2.3.8 Fatty acid

Fatty acid content of samples were analyzed at the Missouri Agricultural experiment station (University of Missouri-Columbia, Columbia, MO) following the procedure of AOAC official method 996.06. Specific type of fatty acid profile was calculated as described below (Benz et al., 2010).

Total saturated fatty acids (SFA) = C8:0 + C10:0 + C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0.

Total mono-unsaturated fatty acids (MUFA) = C14:1 + C16:1 + C18:1 cis-9 + C18:1n-7 + C20:1 + C24:1.

Total polyunsaturated fatty acids (PUFA) = C18:2n-6 + C18:3n-3 + C18:3n-6 + C20:2 + C20:4n-6.

Unsaturated fatty acid (UFA): SFA ratio = (total MUFA + total PUFA)/total SFA.

PUFA: SFA ratio = total PUFA/total SFA.

2.3.9 Amino acid

Amino acid content of samples were analyzed at the Missouri Agricultural experiment station (University of Missouri-Columbia, Columbia, MO) following the procedure of AOAC official method 982.30 E(a,b,c).

2.4 In vitro digestion

To determine the digestibility of feedstuffs, there are different approaches available. No simulated environment can be as accurate as one that occurs in the animal's body. However, in vitro method is preferred nowadays for evaluating feedstuffs, as animal models are very expensive, time consuming and requires high level of technical skills along with a lot of ethical concerns and other logistical limitation involved with it. Most importantly, it is not useful for analyzing single feedstuffs which makes it unsuitable in some cases. On the other hand, in vitro method can be used to screen large set of sample in short period of time with less cost. Most importantly, as live animals are not used, welfare of animal is not compromised which is the big concern these days.

The ground samples were subjected for in vitro digestion. Three step in vitro digestion technique (Boisen and Fernandez, 1997) were used to get the apparent total tract digestibility of nutrients of samples in swine. This technique simulates similar kind of condition occurring in the gastro intestinal tract of swine all the way from pH, temperature and the enzymatic action taking place in the GI tract of swine. The first step involved in this model mimics the activities taking place in the stomach, the second step simulates the small intestine (duodenum, jejunum and ileum) i.e. first and second step mimics activities taking place in the upper gut of swine which

provides information on the apparent ileal digestibility of dry matter, energy and other nutrients, while the third step simulates the activity taking place in the hindgut (large intestine) of swine. To mimic the hind gut this model uses cocktail of fiber degrading enzyme which is commercially available as Viscozyme (Novozymes, Bagsvaerd, Denmark). No actual fermentation takes place in this process as purified enzyme is being used instead of micro-organism.

All the feedstuff went through three step digestion to determine the apparent total tract digestibility of DM, and energy.

1ststep

Briefly, 1gm of sample was weighed in 250ml conical flasks. 50ml of phosphate buffer solution 1 (PBS 1) was added to the sample. PBS 1 (0.1M, pH 6.0) was made by mixing 25ml of 0.1M of sodium monobasic phosphate (Na_2HPO_4) with 175ml of 0.1M potassium dibasic phosphate (KH_2PO_4). 0.1M of Na_2HPO_4 is made by mixing 14.196gm of Na_2HPO_4 in one liter of double distilled water while 0.1M KH_2PO_4 is made by mixing 13.609gm of KH_2PO_4 in one liter of double distilled water. 20ml of HCl solution (0.2M) was poured into the conical flasks. The pH was adjusted to 2.0 by mixing with 1M HCl or 1M NaOH. 1ml of a chloramphenicol (Sigma C-0378, Sigma-Aldrich Corp., St. Louis, MO) solution made by mixing 0.5gm of chloramphenicol in 100ml of ethanol was added to prevent bacterial growth which might take place during hydrolysis. 2ml of fresh pepsin solution was added to the flask. Pepsin solution was made by mixing 0.75 gram of pepsin (Sigma P-0609, 800-2500 units/mg) to 30ml of ultra-pure. Flask was then closed with a rubber stopper and incubated in water bath at 39°C for 2 hour under gentle agitation (50 rpm).

2ndstep

20ml phosphate buffer solution (PBS 2) and 10ml of 0.6M sodium hydroxide (NaOH) was added to the solution in the flask. PBS 2 (0.2M, pH 6.8) was made by mixing 99ml of 0.1M of sodium monobasic phosphate (Na_2HPO_4) with 101ml of 0.1M potassium dibasic phosphate (KH_2PO_4). 0.1M of Na_2HPO_4 is made by mixing 28.392gm of Na_2HPO_4 in one liter of double distilled water while 0.1M KH_2PO_4 is made by mixing 27.218gm of KH_2PO_4 in one liter of double distilled water. The pH was adjusted to 6.8 with 1M HCl or 1M NaOH. 6ml of fresh pancreatin solution made by mixing 3gram of pancreatin (Porcine, grade IV, P-1750 Sigma) to 90ml of ultrapure water was added and hydrolysis was continued for 4 hour under the same conditions.

3rdstep

At the end of the of incubation, 20ml of a 0.2M EDTA (ethylene diamine tetra acetic acid) solution made by mixing 74.448grams of EDTA per 1 liter of ultrapure water was added to the flask, and the pH was adjusted to 4.8 with 30% acetic acid solution (about 1ml of 30% acetic acid). Then 1ml of Viscozyme, a cocktail of fiber degrading enzyme (multienzyme complex made from *Aspergillus aculeatus* containing cellulase, β -glucanase, arabinase, xylanase, mannanase, and pectinase; Novozymes, Bagsvaerd, Denmark) was added, and the flask was incubated at 39°C for 18 hour.

The enzymatic digestion was terminated by adding 10ml of 20% sulphosalicylic acid, and the flask was kept at room temperature for 30 min to facilitate precipitation of undigested soluble proteins. The undigested residue was then collected in a filtration unit using a porcelain filtration funnel lined with pre-weighed filter paper (Whatman no. 54; Whatman Inc., Florham Park, NJ). All the material was transferred with double distilled water to the funnel. The residue, along with the filter paper, was dried overnight at 80°C and weighed back the next day.

For this study, 2 independent in vitro digestion studies were done for agricultural products and agricultural co-products. To get enough digested residues for protein analysis, again one set of in vitro digestion study for agricultural products was conducted. The experimental scheme for the in vitro digestion was as follows:

4 agricultural product × 3 bottles repeated over 4 batches.

4 Agricultural co-products × 3 bottles repeated over 4 batches.

First, three similar sets of the same sample were prepared. Next, four batches of the same sample set were prepared so as to avoid any statistical error that could be caused due to difference in batch composition.

Dry matter, energy and protein digestibility. After three step enzymatic digestion, the residues of agricultural products was oven dried to get constant weight and was used to determine DM, energy and protein content and their in vitro digestibility calculation.

2.5 Calculations

The in vitro digestibility of DM (IVDDM) was calculated as follows:

$$\text{IVDDM} = \frac{\text{Dry weight of sample before hydrolysis} - \text{dry weight of residue}}{W \times \text{DM}} \times 100$$

The disappearance of the other nutrients was calculated using the degradability coefficient of DM and the relative content of individual nutrients in the ingredients and hydrolyzed substrates.

$$\text{In vitro digestibility of gross energy (IVDGE)} = \frac{\text{Gross energy of sample} - \text{Gross energy of residue}}{\text{Gross energy of sample}}$$

$$= [(DM \text{ sample} \times GEs) - (g \text{ residue} \times GER)] / [(DM \text{ sample} \times GEs)$$

$$= [(g \text{ sample} \times DMs) \times GEs] - (g \text{ residue} \times GER)] / [(g \text{ sample} \times DMs) \times GEs]$$

Where,

DMs =% Dry matter of sample

GEs = Gross energy of sample

GER = Gross energy of residue

In vitro digestibility of protein (IVDP) was calculated same as energy digestibility.

2.6 Statistical analysis

The in vitro digestibility of nutrients were compared using MIXED procedure of SAS v9.2 (SAS Institute Inc., Cary, NC), where feedstuffs were treated as fixed factor and batch as random factor. Means were separated using the Tukey method, using pdmix macro of SAS. Differences were considered significant if $P < 0.05$.

Chapter 3: Results

3.1 Nutrient profile of feedstuffs

The results of nutrient profile of agricultural products and Agricultural co-products are presented in tables below.

Table 8. Nutrient profile of agricultural products, % DM basis

Feedstuff	DM	Ash	CP	EE	ADF	NDF	Hemi-cellulose	Starch	GE (Kcal/kg)
Purple sweet potato	42.98	1.95	4.79	2.77	5.68	7.95	2.27	47.02	4134
Okinawa sweet potato	40.83	2.79	5.30	2.04	8.14	9.67	1.53	51.67	4154
Taro	37.43	2.39	8.84	1.87	10.38	11.46	1.08	38.43	4333
Cassava	41.87	4.13	3.73	1.05	6.53	11.30	4.77	60.85	4193

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DM, Dry matter; EE, ether extract; GE, gross energy; NDF, neutral detergent fiber

Dry matter content of agricultural products ranged from 37 to 43%. Purple sweet potato (42.9%) had the highest amount of dry matter while taro (37.4%) had the lowest. Ash content was highest in cassava (4.1%) and lowest in purple sweet potato (1.9%). Crude protein content in all the agricultural products ranged from 4 to 9%, where taro (8.84%) contained highest amount of CP while cassava (3.7%) had the lowest. Ether extract content of agricultural products ranged from 1 to 3%. Purple sweet potato (2.77%) had the highest amount of ether extract and cassava (1.05%) the lowest.

Acid detergent fiber content of agricultural products ranged from 5 to 11%, taro (10.38%) containing the highest and purple sweet potato (5.68%) containing the lowest. Neutral detergent fiber content of all the agricultural products ranged from 7 to 12%, taro (11.46%) possessing the highest and purple sweet potato (7.95%) had the lowest. There was very slight difference in the NDF content of taro and cassava. Both ADF and NDF content was found higher in Taro. The difference between NDF and ADF content in taro was found the least hence it contains less amount of hemicelluloses which is digestible to some extent. Hemicellulose content was highest in cassava (4.77%).

All the agricultural products were starchy tuberous root. The starch content in all the tubers ranged from 38% - 61%, cassava (60.85%) containing the highest and taro (38.43%) containing the lowest.

Not much of difference was found in gross energy content of tubers, it ranged from 4134 to 4333 Kcal/kg, where taro (4333) had the highest amount of energy and purple sweet potato (4134) had the lowest.

Table 9. Nutrient profile of agricultural co-products, % DM basis

Feedstuff	DM	Ash	CP	EE	ADF	NDF	Hemi cellul ose	GE (Kca l/kg)
Macadamia nut cake	91.29	3.74	25.57	11.97	28.00	35.83	7.83	5581
Wheat millrun	96.68	1.80	11.79	4.05	24.16	35.00	10.84	4736
Barley brewers grain	97.09	8.65	15.86	1.79	34.08	42.08	8.00	4270
Okara	35.88	5.17	22.70	13.73	19.73	31.02	11.29	4707

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; GE, gross energy; NDF, neutral detergent fiber

Dry matter content of byproducts ranged from 35 to 92%. Barley brewers grain (97.09%) had the highest amount of dry matter while okara (35.88%) had the lowest. Ash content was found highest in BBG (8.65%) and lowest in WMR (1.80%). Crude protein content ranged from 11 to 26% where MNC (25.57%) contained highest amount of crude protein and WMR (11.79%) had the lowest. Ether extract content ranged from 1 to 13%. Okara (13.73%) had the highest amount of ether extract and BBG (1.79%) the lowest.

The ADF content of the byproducts ranged from 19 - 28%, MNC (28%) possessing the highest and okara (19.73%) the lowest. The NDF content of all the byproducts ranged from 31 - 42%, WMR (42.08%) possessing the highest and okara (31.02%) the lowest. The difference between NDF and ADF content was found to be highest in okara (11.29%), hence it contains highest amount of hemicelluloses which is digestible to some extent. Hemicellulose content of MNC (7.83%) was found to be the least that means it contains less amount of digestible fiber.

Gross energy content was found highest in MNC (5581 Kcal/kg) and lowest in BBG (4270 Kcal/kg)

Linoleic acid (18:2n-6) is an Omega-6 fatty acid; whose concentration was 2.5% in MNC while linolenic acid (18:3n-3) is an Omega-3 fatty acid, whose concentration was 0.2%. The 18C fatty acids are responsible for synthesis of polyunsaturated fatty acids (PUFA). Hence, higher the concentration of these 18C fatty acids in MNC, higher will be the synthesis of polyunsaturated fatty acids (PUFA).

Table 10. Fatty acid profile of macadamia nut cake

Fatty acid profile (expressed as percentage of total fat)	w/w, %
Myristic (14:0)	0.95
Palmitic (16:0)	8.86
Palmitoleic (9c-16:1)	19.64
Margaric (17:0)	0.09
Stearic (18:0)	2.73
Elaidic (9t-18:1)	0.08
Oleic (9c-18:1)	57.43
Linoleic (18:2n6)	2.56
Linolenic (18:3n3)	0.19
Arachidic (20:0)	2.36
Gonodic (20:1n9)	2.77
EPA (20:5n3)	0.00
Behenoic (22:0)	0.81
Erucic [22:1n9]	0.00
DHA (22:6n3)	0.00
Lignoceric (24:0)	0.38
SFA	16.18
MUFA	79.84
PUFA	2.75
PUFA:SFA	0.17

Abbreviations: SFA, Saturated fatty acids; MUFA, Mono-unsaturated fatty acids; PUFA, Polyunsaturated fatty acids;

Table 11. Amino acid profile of macadamia nut cake

Amino Acid	Macadamia nut cake (%DM basis)
Essential amino acid	
Lysine	0.701
Methionine	0.340
Threonine	0.789
Tryptophan	0.208
Phenylalanine	0.745
Histidine	0.449
Valine	0.953
Isoleucine	0.745
Leucine	1.380
Conditionally essential amino acid	
Arginine	2.574
Cysteine	0.602
Proline	1.030
Glutamic Acid	4.360
Tyrosine	0.854
Non-essential amino acid	

Aspartic Acid	2.158
Serine	0.909
Glutamic Acid	4.360
Serine	0.909
Glutamic Acid	4.360

Pigs have specific requirement for amino acids, not for crude protein *per se*. Lysine is the first limiting amino acid in swine, that means if insufficient amount of lysine is included in the diet of pig, then protein synthesis in the animal will not go further beyond the point to which lysine was made available. Dry matter intake of 440lb lactating sow is 11.02lb/day and they require 2.6gm of lysine every day. To meet this requirement a pig would need to eat around 377gm of MNC as lysine content in MNC is only 0.7%.

3.2 In Vitro digestibility

In vitro digestibility of agricultural products and byproducts are presented in Table 12.

Table 12. In vitro dry matter digestibility of agricultural products, %

Feed ingredient	Dry matter digestibility	SEM	P - value
Purple sweet Potato	86.87 ^a		
Okinawan Sweet Potato	81.60 ^b	1.01	<0.0001
Taro	70.32 ^c		
Cassava	82.12 ^b		

Abbreviations: SEM, standard error of mean

In vitro dry matter digestibility among agricultural products was significantly higher ($P < 0.001$) in PSP (86.87%) than taro (70.32%), whereas others were in-between.

Table 13. In vitro gross energy digestibility of agricultural products, % DM basis

Feed ingredient	Energy digestibility	SEM	<i>P</i> - value
Purple sweet potato	87.56 ^a		
Okinawan sweet potato	82.39 ^b	1.11	<0.0001
Taro	64.99 ^c		
Cassava	83.18 ^b		

Abbreviations: SEM, standard error of mean

In vitro gross energy digestibility among agricultural products was significantly higher ($P < 0.001$) in PSP (87.56%) than taro (64.99%) whereas others were in-between.

Table 14. In vitro crude protein digestibility of agricultural products, % DM basis

Feed ingredient	Protein digestibility	SEM	<i>P</i> - Value
Purple sweet potato	76.39 ^a		
Okinawan sweet potato	63.12 ^b	2.01	<0.0001
Taro	63.17 ^b		
Cassava	74.47 ^a		

Abbreviations: SEM, standard error of mean

In vitro crude protein digestibility among agricultural product was significantly higher ($P < 0.05$) in PSP (76.39%) and cassava (74.47%) than taro (63.17%) and OSP (63.12%).

Table 15. In vitro dry matter digestibility of agricultural co-products

Feed ingredient	Dry matter digestibility (%)	SEM	P - Value
Macadamia nut cake	75.71 ^a		
Wheat mill run	69.90 ^b	0.86	<0.0001
Barley brewers grain	61.33 ^c		
Okara	74.10 ^a		

Abbreviations: SEM, standard error of mean

In vitro dry matter digestibility among co-products was significantly higher ($P < 0.05$) in MNC (75.71%) and okara (74.10%) than BBG (61.33%).

Table 16. In vitro gross energy digestibility of agricultural co-products

Feed ingredient	Gross energy digestibility (%)	SEM	P - Value
Macadamia nut cake	71.40 ^a		
Wheat mill run	53.09 ^b	1.51	<0.0001
Barley brewers grain	43.04 ^c		
Okara	66.23 ^a		

Abbreviations: SEM, standard error of mean

In vitro gross energy digestibility among co-products was significantly higher ($P < 0.05$) in MNC (71.40%) and okara (66.23%) than BBG (43.04%).

Chapter 4: Discussion

Market availability of traditional feed ingredients is variable and the costs of these ingredients has soared up and are expected to increase continuously due to limited production as well as competition among food (humans), feed (livestock) and fuel. Farmers and nutritionists are always looking for cost-effective feedstuffs for animal feeding to be competitive in the market place. There are some agricultural products and agricultural co-products available, but these are not used in routine animal feeding program as nutritional value and digestibility information of these potential feedstuffs are either unavailable or available to a very limited extent. Nutritional value of feedstuff is affected by several factors such as climatic condition of the place where these feedstuffs are grown, soil quality, type and varieties of seed used for cultivation, processing method involved etc. Hence, this study was intended to evaluate the nutritional value and digestibility of some of agricultural products and agricultural co-products available in Hawaii.

4.1 Nutritional composition

Crude protein content of both purple sweet potato (4.8%) and Okinawan sweet potato (5.3%) was slightly lower (6.4%) than reported by Dominguez (1992) but slightly higher (4.4%) than reported by Noblet et al. (1990). Macadamia nut cake and okara have the potential to be used in an animal feed, as the CP concentration in these feedstuffs are fairly high (around 22-25%). Although both of these have almost half of the CP content of soybean meal, still it is advantageous over cereal grains like corns, which contains almost half or even lower CP in general. The CP content of MNC used in this study was found to be 25.8% which is higher than 20.9 % as reported by Skenjana et al. (2011), 14% by Van Ryssen et al. (2014) and 19.5 % by Acheampong et al. (2008). Quality of kernels used and oil extraction process applied might be

the possible reason for this variation observed. CP content of okara in this study was found to be 22.7%. However, Li et al. (2012) found varying level of CP content in okara, ranging from 15.2 to 33.4%. The CP content of MNC and okara used in this study was found to be 25.6% and 22.7%, respectively which is sufficient enough to serve as a good protein source in swine diets.

Quality of protein is always the major concern while using agricultural co-product as various factors affects the nutritional quality of protein like amino acid profile and its digestibility and anti-nutritional factors present in the feedstuff. Heat treatment to which the feedstuff is subjected to while processing might play important role in degrading the quality of protein.

Energy content in fat is 2.25 times more than that of carbohydrate and protein. Hence, MNC and okara can also be viewed as a potential source of energy along with protein because of high fat content in MNC and okara (11.97% and 13.73% ether extract, respectively). This is also reflected by their GE content (5581 and 4707 Kcal/kg, respectively).

Ash content in cassava was higher than other tubers, while BBG had the highest ash content among the co-products. Higher ash concentration does indicate higher amount of minerals present in it but does not tell anything about the concentration of any individual mineral. Although minerals are not digested by the animals, but some macro minerals and micro minerals plays important role in maintaining health of animals. Contamination with soil while harvesting can also result in higher ash content in Cassava.

Crude fiber content of MNC in this study was found to be 25.26% which is similar (24.9%) to that reported by Acheampong et al. (2008) but relatively lower than that reported by Van Ryssen et al. (2014) and Skenjana et al. (2011), i.e. 36.5 and 29.0%, respectively. The ADF

content of PSP (5.6%) in this study was similar to that observed by Dominguez (1992) and Noblet et al. (1990) who reported it to be 5.5% and 4.2%, respectively but ADF content of OSP (8.14%) observed in this study was slightly higher than those previous studies. The NDF content of the MNC used in present study was 32.7% which is comparatively lower than that reported by Acheampong et al. (2008) and Skenjana et al. (2011) they found it to be 51.8 and 49.8% respectively. The ADF fraction contains cellulose and lignin, of which lignin is almost indigestible, while digestibility of cellulose varies. ADF concentration in MNC in the present study was 27.9% which is much lower than reported by Skenjana et al. (2011) i.e. 40.0% and Acheampong et al. (2008) i.e. 41.5%. However, lignin (16.0%) and hemicellulose (7.8%) concentration of MNC in the present study was similar to that (15.0 and 9.8%, respectively) reported by Skenjana et al. (2011). This high fiber fraction of MNC observed by other researchers might be because of oil extraction process; the samples collected might have been from the oil extraction process applied on low grade macadamia kernels, or the sample might have contained higher concentration of macadamia shell pieces, which is not clear in those papers. Concentration of lignin observed by most of the researchers ranged from 14 to 17 %, including in the present study. Though there are still unresolved mechanisms, lignin inhibits digestion of plant cell wall components (Morrison, 1983). The higher the lignin, lower will be the digestibility as lignifications within plant species is negatively associated with NDF digestibility.

This difference in the nutrient profile of co-products might be because of the difference in the processing technique to which the feedstuff is subjected to, also because of the quality of nuts used for oil extraction and grain used for brewing, mashing time and quality of adjuncts added in brewing process (Santos et al., 2003). Also, there was potentially leaching of nutrient due to

variation in the storage condition like temperature, humidity, rain etc. Low nutritive value of MNC observed by Skenjana et al. (2011) was mainly because of the presence of nuts and hulls in MNC that the researchers analyzed while this was not the case in the present study.

Amino acids are the component that makes up protein. Pigs have specific requirement for this amino acids, not for crude protein *per se*. Essential amino acids are not synthesized by animal, thus need to be supplied through the diets. Lysine is the first limiting amino acid in pigs, that means if insufficient amount of lysine is included in the diet of pig, then protein synthesis in the animal will not go further beyond the point to which lysine was made available. Potential growth of swine is being limited by this limiting amino acid. Thus, balancing lysine is of vital importance in pig diets. Requirement of lysine varies with age and stage of production. While formulating diets, lysine requirement is calculated first, and then other individual amino acids are supplied as ratios to lysine or as percentage of lysine. This is also known as ideal protein ratio. Methionine, threonine and tryptophan are respectively second third and fourth limiting amino acid in diet of swine. If lysine (first limiting amino acid in pigs) is not supplemented but methionine and threonine (second and third limiting amino acid) is supplemented, then growth performance of pigs will be suppressed as they do not satisfy the amino acid requirement. In other words, as long as first limiting amino acid is not fulfilled, supplying other amino acids (protein) is of no use as animal cannot utilize other amino acids. Similarly, when first limiting amino acid requirement is fulfilled supplying third amino acid without satisfying the second is not going to improve the animal performance. Hence amino acid should be added in right sequence as per the requirement of pig.

Table 17. Amino acid profile of MNC as compared to nutrient requirement of swine (NRC, 2012)

Amino Acid	Macadamia nut cake (%DM basis)	110 lb pig (4.4 lbDMI/day gm/day)	440.9 lb Gestating Sow (4.4 lbDMI/day gm/day)	440.9 lblactating sow (11 lb DMI/day gm/day)
Essential amino acid				
Lysine	0.701	1.340	1.850	2.640
Methionine	0.340	0.370	0.490	0.540
Threonine	0.789	1.830	2.370	3.020
Tryptophan	0.208	0.510	0.580	0.740
Phenylalanine	0.745	1.210	1.330	1.900
Histidine	0.449	0.470	0.640	1.050
Valine	0.953	1.580	2.010	3.500
Isoleucine	0.745	1.170	1.580	2.060
Leucine	1.380	1.670	1.920	2.840
Conditionally essential amino acid				
Arginine	2.574	0.730	0.910	2.040
Cysteine	0.602			
Proline	1.030			
Glutamic Acid	4.360			
Tyrosine	0.854			

Non-essential amino acid

Aspartic Acid	2.158
Serine	0.909
Serine	0.909
Glutamic Acid	4.360

Lysine concentration of MNC in this study (0.70%) was greater than 0.64%, reported by Phosa et al. (2009), still amino acid requirement of swine will not be satisfied by supplementing diet containing 100% MNC, unless excess amino acid is supplied (NRC, 2012). Dry matter intake of 440lb lactating sow is 11 lb/day and they require 2.6gm of lysine every day. To meet this requirement a pig would need to eat around 377gm of MNC as lysine content in MNC is only 0.7%. According to NRC (2012), amount of amino acid utilized by swine is lower than that contained in the feedstuff. In other words, 100% of amino acid present in MNC is not available to animal as MNC is not 100% digested. Bioavailability of amino acids in swine is measured more accurately by ileal digestibility than by total tract digestibility as absorption of amino acid only takes place in small intestine. This study only presents the amino acid content in MNC but further in vivo trial is needed to determine standardized ileal digestibility which would help in formulating the diet. Balancing dietary amino acid is the most important factor which affects the efficiency of utilization of protein in swine. Hence, before formulating any diet based on MNC, deficient amino acid should be supplied especially lysine, methionine and threonine.

Energy of fat is stored as tryacylglycerol. Energy contribution of fats is 2.25 times more than what is provided by carbohydrates or protein of same amount (Schingoethe, 1991). Besides providing energy in concentrated form, dietary fat also plays vital role as a source of essential

fatty acids for animals. Linoleic, linolenic and arachidonic acids are the essential fatty acids. These essential fatty acids contains more than one double bond, hence are called unsaturated fatty acids. Most of the metabolically important fatty acids are synthesized from 18:2n-6 and 18:3n-3 fatty acids and these fatty acids are also considered as parent compound. Linoleic acid (18:2n-6) is an Omega-6 fatty acid while linolenic acid (18:3n-3) is an Omega-3 fatty acid. Arachidonic acid is synthesized from linoleic acid where as linolenic acid is responsible for synthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Addition of PUFA (omega 3 and omega 6 fatty acids) in the diet of pig would help in increase of arachidonic acid, EPA and DHA in the tissues (Leskanich *et al.*, 1997) as concentration of fatty acid in feed proportionately affects the concentration of fatty acid in the body tissues (Bee *et al.*, 2002). These 18C fatty acids are responsible for synthesis of polyunsaturated fatty acids (PUFA). Hence, higher the concentration of these 18C fatty acids in MNC, higher will be the synthesis of PUFA. There is high demand for food containing high PUFA. Higher concentration of omega 3 and omega 6 fatty acid in pig's diet will cause higher deposition of this fatty acid in tissues of animal as fatty acid intake is proportionately related to fatty acid deposition. PUFAs are very important for swine as they provide fluid nature to the cellular membrane. In the absence of PUFAs, the membrane would get instable as more saturated fatty acids and less fluid will be included. PUFA are precursor for eicosanoids which affects immunity. Eicosanoids derived from n-6 fatty acids is inversely related to those derived from n-3 fatty acids. Hence, ratio of n-3 and n-6 affects the net effect of eicosanoids.

Diet containing higher concentration of PUFA is more vulnerable to oxidative rancidity than the diet containing higher concentration of monounsaturated fatty acid (MUFA). Rancidity results in degradation of pleasant taste as well as reduction in the shelf life of meat. Inclusion of

higher concentration of unsaturated fatty acids in the pigs diet might result in development of unpleasant flavor in the edible tissues of meat as well as softening of fat deposit (Chow, 1980), hence reducing the quality of meat and raising a big question mark on the acceptability of such animal products. On the other hand, PUFA has been claimed to have several health benefits. Reducing serum cholesterol, reducing platelet aggregation etc are some of anti-inflammatory properties exhibited by PUFA.

4.2 In vitro digestibility of nutrient

The dry matter digestibility of purple sweet potato (86.8%) was highest among all feedstuffs tested. This can be attributed to lowest NDF and ADF content in purple sweet potato. The ADF is inversely related to digestibility, i.e. higher the ADF content in the feedstuff, lower the digestibility. Fiber content in taro was comparatively higher than other tubers. Difference between the NDF and ADF content was also found to be least in taro i.e. it contained least amount of hemicelluloses hence digestibility of taro was least digestible feedstuff among the tubers. Hemicelluloses are relatively better utilized than cellulose in the intestine of pig. Cassava had higher amount of hemicellulose (4.77%), hence its digestibility (82.12%) was better than that of taro. Lower digestibility in Taro might be because of higher amount of fiber present in outer skin of taro. Whole taro was used without peeling the outermost skin.

Although energy content in taro was higher than other tubers studied, the amount of energy which is actually utilized in the body of swine is very less, it's just 64.99%. Energy content in purple sweet potato is highly digestible. Less amount of purple sweet potato will be enough to meet a particular energy requirement of swine, but large amount of taro will be required to fulfill the same energy requirement as its energy digestibility is lowest. Relative growth capacity of young piglets is highest but their feed intake capacity is low, so purple sweet

potato can serve as potential energy dense diet for piglets, so that they can eat less and obtain the appropriate requirement. On the other hand, energy requirement of sows in the gestation period is less but still are capable of eating more than they actually need. For such sows, taro having low energy digestibility can serve as potential source of energy as the energy which they will actually digest will be less. Starch content in cassava (60.85%) was highest while taro (38.43%) possessed least amount of starch. High amount of fiber and low amount of starch can be the limiting factor in the use of taro. However, it can serve as important source of energy in the places where it is found in large quantity and its digestibility can be increased by peeling off the outermost skin.

Although protein content in taro (8.84%) and okinawan sweet potato (5.30%) was higher than that of cassava (3.73%) and purple sweet potato (4.79%), the amount of protein which is actually digested by animal is least in them. Protein digestibility of purple sweet potato and cassava was significantly higher than that of taro and okinawan sweet potato.

In vitro DM digestibility of okara and MNC was fairly high, around 74 and 75%, respectively. It implies that 74% of DM present in okara and 75% of DM present in MNC are available upon its ingestion. Not only for swine, MNC has also been found suitable for other livestock like high producing ruminants and other monogastric animals like poultry. No negative effect on performance was seen when MNC was added in the poultry diet at an inclusion level of 10% (Phosa et al., 2004a). It implies that MNC can serve as a relatively cost-effective alternative feedstuff which has the potential of replacing maize by 10% in the diet of poultry (Phose et al., 2004a). Phosa et al. (2004b) recommended inclusion of MNC at the rate of 10% in diets of both layer and broiler chicken. All the agricultural co-products studied showed a potential to be used in swine diet but these feedstuffs need to be subjected to animal trials to have a better idea about

in vivo digestibility and voluntary feed intake by animals. A thorough study on anti-nutritional factors present in these agro-industrial byproduct need to be performed to get a better idea about the extent to which the concentration of ANF can affect palatability (thereby feed intake) and digestibility.

The major limitation in the use of agricultural co-products as an animal feedstuff is the variation in their nutritional composition. One of the possible ways to minimize the effect of variation is by routine analysis of each batch coming out from the processing industry. There are high chances of getting poor quality MNC because of contamination with high concentration of shell in different batches. These shells are indigestible, thus increases indigestible fraction in MNC. Hence, inclusion of this kind of contaminated MNC in the diet can lead to poor performance (lower growth rate and poor feed conversion efficiency) of animals. Similar is the case with barley brewers grain as it depends upon the quality of barley and adjuncts used during brewing, okara where cultivar of soybean used, production method applied and amount of water phase extracted from ground soybean affects the chemical composition. Thus, every batch from the processing industry should be analyzed for their nutritional profile before using in animal diet formulation.

Another limitation of using MNC is the higher possibility of this products being oxidized and getting rancid because of its high and variable oil content. Environmental factors such as temperature and humidity plays important role in causing rancidity of these products. Storing MNC in cool areas can prevent this product from going rancid sooner. However, Oxidation of fats can also be prevented by including antioxidants in feed.

Most of the phosphorus present in MNC is in the form of phytate, which is an ANF present in MNC. Phytic acid not only reduces the phosphorus availability but also acts as

chelating agent and chelates calcium, magnesium, iron and zinc; thus inhibits absorption of these minerals. Oxalate is an ANF present in taro, cynogenic glycosides in cassava, trypsin inhibitor in okara. The ANF are factors which affects the health of animal, retards the growth either by themselves or through their metabolite by interfering with the utilization of available nutrients present in the diet (Huisman and Tolman, 1992). Poor animal performance might be an indicator of presence of such ANF. However, some ANF can be deactivated by processing techniques like trypsin inhibitor is inactivated by heating.

Okara and Barley brewers grain are high in moisture content, so they are unstable and are vulnerable to microbial activity which can degrade the quality of these feedstuffs and spoil it within a week or so (Taruna et al., 2002; Stojceska et al., 2008; Robertson et al., 2010). Drying can help to preserve these feedstuffs, and also reduce transport and storage cost as drying reduces the volume (Santos et al., 2003). Bartolome et al. (2002) proposed three method of drying; oven drying, freezing and freeze drying. Freezing such a huge volume is not feasible; freeze drying is expensive and economically unsustainable. Oven drying should be conducted at a lower temperature, generally below 60oC to prevent generation of unpleasant flavor. Santos et al. (2003) recommended pressing and reducing the moisture content below 60% and then drying to reduce moisture content below 10%.

Okara is relatively cheaper than soybean meal, high in protein and is palatable. This can be used as a source of protein in the pig's diets. Various processing techniques can be used to improve nutritional quality and flavor of agricultural co-products like, enzymatic treatment, fermentation, extrusion, micronization etc. Mo et al. (2007) found increase in CP content of okara when it was allowed to ferment for 3 days by *Aspergillus oryzae*, *Aspergillus niger* and *Saccharomyces cerevisiae*. Fiber in co-products can be degraded into low molecular weight

carbohydrates by molds during fermentation, which can be utilized by yeast to synthesize protein. Use of enzymes has been found to be effective in utilizing fiber to some extent and improve nutritive value of feedstuffs. However, cost involved in enzyme supplementation should be considered while formulating diets using agricultural co-products.

4.3 Conclusion and Recommendations

Agricultural products are rich in energy and other nutrients. In vitro digestibility of DM, GE, and CP varies among these products. Purple sweet potato had the highest in vitro digestibility of all nutrients, while taro had the lowest. Cassava and okinawan sweet potato had similar in vitro DM and GE digestibility. However, in vitro CP digestibility was higher in cassava than in okinawan sweet potato.

Agricultural co-products are rich in fiber, protein as well as other nutrients. In vitro digestibility was higher in okara and macadamia nut cake, whereas digestibility of barley brewers grain and wheat mill run was almost similar.

Although fiber, moisture and anti-nutritional factor might be some limitation in the use of these agricultural products and agricultural co-products but their potentiality cannot be ignored. Agricultural products can be used as partial substitute of common energy ingredients in pig diets, especially for subsistent farming system where these products are grown and are widely available whereas agricultural co-products can also be used to replace traditional feed ingredients to some extent and can serve as potential source of protein. Processing can cause variation in the nutritional content; hence, nutrient evaluation of these feedstuffs should be carried out before they are supplemented in the diet of swine. Balancing essential amino acid, especially limiting amino acids has to be considered while formulating swine diets based on these alternative feedstuffs. Diet should also be balanced for energy and other nutrients to meet the nutritional requirement of pigs by supplementing other sources.

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