Perilla Mint (*Perilla frutescens*): An Alternative Animal Feed to Enhance Omega-3 Fatty Acids in Meat and Eggs as Functional Food

งาขี้ม้อน: ทางเลือกอาหารสัตว์เพื่อเพิ่มปริมาณกรดไขมันโอเมกาสามในเนื้อสัตว์และไข่สำหรับเป็นอาหารเพื่อสุขภาพ

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บทคัดย่อ: สุขภาพมนุษย์กลายเป็นเรื่องที่น่าวิตกกังวลจากตัวเลขของจำนวนผู้ป่วยที่เป็นโรคหลอดเลือดหัวใจทั่วโลกที่กำลังเพิ่มสูงขึ้น การบริโภคอาหารในลักษณะของอาหารเพื่อสุขภาพ (functional food) โดยเฉพาะอย่างยิ่ง omega-3 ซึ่งเป็นทางเลือกหนึ่งในการรักษาสุขภาพโดยการบริโภคสารอาหารที่ดีต่อสุขภาพ จากการรายงานข้อดีของกรดไขมันโอเมกา (omega) 3, 6 และ 9 ในด้านการลดความเสี่ยงของการเกิดโรคหลอดเลือดหัวใจได้เป็นอย่างดี omega-3 เป็นกรดไขมันที่ทราบกันดีว่ามักพบในปลาทะเล แต่เมื่อไม่นานมานี้มีนักวิจัยรายงานว่าพบว่าในงาขี้ม้อน (*Perilla frutescens*) เป็นแหล่งกรดไขมัน omega-3 ที่มีศักยภาพสูงเช่นกัน โดยเฉพาะเมล็ดงาขี้ม้อนมีการรายงานว่าพบองค์ประกอบของ omega-3 สูง และเมื่อใช้เมล็ดงาขี้ม้อนและกากงาขี้ม้อน ร่วมในอาหารสัตว์ เช่น สุกร และไก่ พบว่าสามารถเพิ่ม omega-3 ในเนื้อสุกร ไก่เนื้อ และไข่ได้ และข้อดีของงาขี้ม้อนยังสามารถลดต้นทุนการผลิตสัตว์ ได้เนื้อที่มีคุณค่าสูงกว่าแหล่งโปรตีนในอาหารสัตว์ทดแทนแหล่งโปรตีนอื่นเช่น กากถั่วเหลือง นอกจากนี้เมล็ดงาขี้ม้อนยังมีสารยับยั้งการทำงานของเอนไซม์ทริปซินต่ำกว่ากากถั่วเหลืองอีกด้วย ซึ่งถือว่าเป็นข้อดีในการนำมาใช้เป็นอาหารสัตว์

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Abstract: Human health is an increasing concern reflected, for example, by a rising number of patients with diseases like coronary artery disease (CAD) worldwide. The consumption of functional food, which is rich in omega-3 fatty acid is one possibility to improve health through a better nutrition. In the literature, benefits of omega-3, -6 and -9 fatty acids in terms of reducing the risk for CAD are well described. Omega-3 is known to be rich especially in marine fish. Recently, researchers reported the plant perilla mint (*Perilla frutescens*) as another potential source of this fatty acid. Particularly, the seeds of this plant have been reported with high omega-3 contents. When used as feed in swine and chicken, perilla seed and meal were found to increase omega-3 fatty acid contents in meat and other animal products such as eggs. An advantage of perilla mint is that production costs are much lower than for other crops such as soybean. Furthermore, perilla seeds have low levels of trypsin inhibitors, which make it suitable for use as animal feed.

Keywords: Perilla mint, carcass characteristic, meat quality

Introduction

In 2013 and 2014 consumption rate of eggs in Thailand was 167.8 and 177.3 eggs per person per year, respectively. During 5 years (2010-2014) consumption rate of egg in Thailand increased by 4.77% annually (Office of Agricultural Economics, 2014), mainly due to the fact that eggs are available at lower costs than other protein sources. Hence, improving nutrition through the consumption of eggs is an important issue for good health. Meat and poultry meat are 5 to 15 folds lower in omega-3 contents than seafood. Pork consumption of Thai people is further and further increasing and in 2013 Thai people consumed 14.0 kg per person per year, which increased to 14.88 kg in 2014. Poultry meat consumption in Thailand 2013 was 15.5 kg per person per year, increased by 7.73% in 2014 to 16.7 kg (Office of Agricultural Economics, 2015). This emphasizes the importance of egg and meat consumption as important source of protein for humans. However, it can cause negative effects in humans because of several compounds such as saturated fatty acids, cholesterol, toxic contaminations, nitrate-nitrite preservatives and antibiotic. For example, saturated fatty acids and cholesterol contained in eggs and meat are causative for several diseases in human health.

Nowadays, the quality of animal products, especially from a human health perspective, is of increasing importance. One reason is the steady increase of coronary artery disease (CAD), the most important cause of cardiovascular mortality worldwide, with over 4.5 million deaths occurring in the developing countries alone and 29% of Patients in Thailand were cardiovascular disease which is the highest than other disease (WHO, 2014). One of the most important risk factors for CAD is the nutrition. The fatty acid composition, especially those of the saturated fatty acids, represents a major risk factor. Consuming food rich in saturated fatty acids (i.e., meat, butter, and cheese) increases the amount of low density lipoprotein (LDL) in the blood but also increases high density lipoprotein (HDL the "good" cholesterol) and decreases triglycerides (Mozaffarian *et al.,* 2010). The main functional difference between HDL and LDL is where they transport cholesterol to. LDL takes cholesterol from liver to the cells while HDL carries cholesterol excess from the blood stream into the liver for removing. The main structural difference is that LDL particle is 50% cholesterol and 25% protein while an HDL particle is 20% cholesterol and 50% protein (Harvey and Ferrier, 2011). This in turn carries cholesterol to tissues, including the heart arteries, and is thus causative for atherosclerosis (LDL forms plaque in vessels and blocks the blood system) causing CAD (Sudheendran *et al*., 2010). The main sources of saturated fatty acids in the food are animal products, such as milk, meat, and eggs. Also, several plant products such as chocolate and cocoa butter, coconut, and palm kernel oil are rich in saturated fatty acids. Consuming too much saturated fatty acid lead to CAD, heart stroke, heart disease, blood pressure, type 2 diabetes and many other diseases (de Souza *et al.,* 2015). Numerous previous studies reported positive effects of unsaturated fatty acids, namely α-linolenic acid (omega-3) and linoleic acid (omega-6) (Lecarf, 2009), on human health by decreasing the risk for CAD and other diseases. Therefore, consumers worldwide are increasingly demanding food products that are rich in omega-3 fatty acids. Increased levels of omega-3 in eggs were realised by supplementation or adding several sources of omega-3 such as flaxseed, fish oil and microalgae (Fraeye *et al.,* 2012) in laying-hen diets. Thus, enhancing omega-3 levels in eggs and meat though the use of agricultural by-products such as perilla meal, which is rich in omega-3, may be one of the possibilities to produce healthy food for the consumer. It is well known people in Thailand consume rice are main course, that is different from westerner, their main course is meat. Therefore, meat enhance with omega-3 is an alternative for meat lover in Thailand and other countries which are like to meat.

Perilla mint is known by many names, including Chinese basil, purple mint, rattlesnake weed and beefsteak plant (Glenn *et al*., 2010). The plant can be found on pastures in Southern India, South Korea, northern Thailand, China, Japan, Vietnam and Taiwan (Hyun *et al.,* 2014). Perilla mint was found in the northern part of Thailand such as Chiang Mai, Chiang Rai, Phayao and Mae Hong Son, cultivated area are 1,360 acre can produce perilla seed 272,000 kg (Chairaungyost, 2012). As a traditional oilseed, it is belonging to the Family lamiaceae. It is widely used in households (CSIR, 1966) for sprinkle on top of soups, salads, sushi and pickles or garnishes (Li *et al*., 2008; Meng *et al*., 2009; Ha *et al*., 2012). The plant is an annual mint and blooms once a year in cool season, thus only one annual harvest is possible (Ampanchai *et al.,* 2008). The different parts of the plant are used for different purposes: leaves are known for their antioxidant and phenolic compounds, while seeds are rich in protein and fat. The tribal population in northeast India have been consuming perilla seed as well as oil without any negative effects being reported (Longvah *et al*., 2000).

In the following, this article reviews the present knowledge on perilla mint when used as animal feed in order to improve the quality of meat and other animal products in terms of increasing the omega-3 fatty acid content.

Chemical composition *of Perilla frutescens*

As generally in plants, the different parts of perilla mint differ largely in their chemical composition. The protein and fat content of its seeds was 18.1 and 40.1%, respectively (Joshi *et al*., 2015). Longvah and Deosthale (1991) reported similar values (protein 17%, fat 51%). Most vegetable oils are good sources of linoleic acid, but only few vegetable oils contain significant amounts of α-linolenic acids. Among them, perilla oil which have the highest contents of α-linolenic acid (omega-3), amounting nearly 57% (Longvah and Deosthale, 1991), linoleic acid (omega-6) 14% and oleic acid (omega-9) ranging from 14-23% (Asif, 2011). Contents are even higher than in fish oil (Kim and Choi, 2005). In addition, the content of saturated fatty acids, namely palmitic acid (5-7%) and stearic acid (0-1%) is lower than in other sources of oil (Table 1). When taking the FAO/WHO/UNU (1985) pattern of essential amino acid requirement for infants as the reference (Table 2), the protein scores of egg and cow milk are 100, while perilla whole seed and perilla without hull are 63 and 66, respectively. The amino acid lysine was the limiting amino acid in both cases. The amino acid score of perilla whole seeds and kernel were comparable to sunflower (61) and peanut (69), both of which also have lysine as the limiting amino acid (Bodwell and Hopkins, 1985). Consequently, perilla seeds can be considered as a better source of essential amino acids than soybean seed or sunflower meal (Table 2).

Perilla meal is a by-product of perilla seed after defatting. Protein contents are about 40.1% with 39% of the total content being essential amino acids. These levels are somewhat lower than in most animal products (beef 52, egg 55 and cow milk 54%) (Longvah and Deosthale, 1998). Perilla meal has compared to other plants such as soybean meal, sunflower meal, canola meal, cotton seed meal and sesame meal similar protein levels. It is therefore a good protein source for animal diets and good for eliminating agricultural by-products (Kusanteay and Uriyapongson, 2016).

Benefits of omega-3 fatty acids on human health

Mono- and polyunsaturated fatty acids are also known as omega fatty acids. Omega-3 fatty acids are polyunsaturated fatty acids where the first

Table 1. Fatty acid composition from different sources of oil (% of fatty acid)

Fatty acid	Beef tallow	Corn oil	Perilla seed	Perilla oil	Fish oil	Sunflower oil	Soybean oil	Palm oil
Saturated fatty acid								
Myristic (C14:0)	3.96	-	-	-	-	-	-	0.88
Palmitic (C16:0)	28.36	11.85	5-7	6.80	25.66	5.73	12.0	46.16
Stearic (18:0)	20.87	-	0-1	2.07	6.65	3.47	5.0	5.07
Arachidic (20:0)	-	0.54		-	-	0.54	1.0	0.41
Unsaturated fatty acid								
Palmitoleic (16:1)	2.79	-		-	5.50	0.02	-	0.13
Oleic (18:1)	44.02	28.94	NA	15.23	13.82	26.45	19-34	37.18
Linoleic (18:2)(n-6)	-	57.29	14	2.07	1.62	62.33	48-60	9.08
α-linolenic (18:3) (n-3)	-	0.93	57	61.30	1.08	-	2-10	0.36
Eicosapentaenoic acid (20:5) (n-3)	-	-	NA	-	32.72	-	-	-
Docosahexaenoic acid (22:6)(n-3)	-	-	NA	-	6.47	-	-	-
Source	Kim *et al.* (2005)	Kim and Choi (2005)	Peiretti (2011)	Kim *et al.* (2005)	Kim and Choi (2005)	Rosa *et al.* (2009)	Su *et al.* (2015)	Su *et al.* (2015)

Table 2. Essential amino acids from different sources (mg/g protein)

Essential amino acid	EAA recommendation for human (FAO/WHO/UNU, 1985)	Perilla seed	Soybean seed	Sunflower meal	Peanut meal	Egg	Cow milk
Histidine	22	31	12	8.8	6	22	27
Isoleucine	18	41	21.6	15.2	8.3	54	47
Leucine	25	62	36.2	21.9	15.3	86	95
Lysine	22	37	29.6	14.6	8.5	70	78
Methionine+ Cysteine	29	26	13.2	15.9	5.9	57	33
Phenylalanine+ Tyrosine	34	55	40	25.2	21.9	93	102
Threonine	30	34	19.3	12	8.1	47	44
Tryptophan	7	11	6.5	-	2.3	17	14
Valine	31	35	22.2	17.5	9.9	66	64
Source	FAO/WHO/UNU (1985)	Longvah and Deosthale (1998)	ENV/JM/MONO (2001)	Rosa *et al.* (2009)	Settaluri *et al.* (2012)	Longvah and Deosthale (1998)	Longvah and Deosthale (1998)

double bond is located at the third carbon from the methyl end of the fatty acid chain. Marine animals are rich in the two long-chain omega-3 fatty acids docosahexaenoic acid (DHA; 22:6) and eicosapentaenoic acid (EPA; 20:5). Plants usually contain the omega-3 fatty acid, α-linolenic acid (18:3) as omega-3. Several studies have reported that α-linolenic acids and its derivatives are beneficial for the human health and fulfill essential physiological functions (Tinoco, 1982; Zollner, 1986; Crawford, 1987; Budowski, 1988; Neuringer *et al.,* 1988). The consumption is, for example, known to lower blood lipid concentration, reduce the risk for thrombosis, prevent Alzheimer's disease, decrease the risk for vascular disease and sudden death from ventricular fibrillation and tachycardia (Azin and Behnood, 2014). Omega-3 fatty acids fulfill neuroprotective actions in Parkinson's disease and exhibit protective effects in Alzheimer's disease, too (Asif, 2011). Omega-3 fatty acids prevent the neurotoxin-induced decrease of dopamine that normally occurs. Therefore, the dopamine system that was disrupted will cause Parkinson's disease (Talbott and Hughes, 2006). α-linolenic acid is the precursor of n-6 and n-3 polyunsaturated fatty acid (PUFA) families, linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3) are finally transformed to EPA and DHA in vivo (Weizhuo *et al*., 2013). The ratio of linoleic acid and α-linolenic acid in the diet is important for converting into long chain polyunsaturated fatty acids, including EPA and DHA (Diwakar *et al*., 2008). As above-mentioned, these affect the development of the human brain. Both EPA and DHA have several effects on inhibiting development of atherosclerosis, decrease triglyceride level and raise levels of HDL (high density lipoprotein “good cholesterol”) in blood plasma, but do not lower plasma cholesterol levels (Conner, 2001). Moreover, the impact of them may be primarily in preventing or relieving arrhythmia. Harris *et al.* (2009) reported cardiac mortality to be reduced by about 35% by modest EPA+DHA consumption. Although, omega-3 fatty acid, DHA and EPA have many advantages for the health but if consumed excessively (normally over 3 g per day), especially in patient that at the same time consume aspirin or warfarin, will increase bleeding or hemorrhagic stroke (only in cases of very large doses) and reduced glycemic control among diabetics (Lewis, 2008; Kromann and Green, 1980). According to the Food and Nutrition Board 2015 (Ornish, 2016) the acceptable intake for n-3 fatty acid is 1.6 g/day and 1.1 g/day for men and women, respectively (Asif, 2011).

Use of perilla mint as animal feed

Perilla mint was used as protein source but in their seed compound with a lot of oil. Fats and oils are a chemically diverse group of compounds. They have the highest average energy density among all macro nutrients. Therefore, fat is an important energy in diet and improve palatability and texture of diet in addition decrease dustiness and easy to make pellets. But they have limit level to use in animal diet such as not over 7% in swine diet because it causes flow ability problems preclude with swine (Shannon, 2016), in poultry not over 4% because it effect to decrease digestibility (Tancharoenrat, 2014). Perilla mint is not have only benefit but also have negative effect, some research reported about toxicity of perilla mint especially, perilla’s leaves is important problem in cattle poisoning that cause pulmonary edema (Brenner, 1993) because perilla ketone substance which found in leaves stem flower and root (Wilson *et al.,* 1977; Glenn *et al.,* 2010). Planta are most toxins when farmer cut and dried for hay late in summer, during flowering and seed production because flowering structure are the most dangerous but the ketone was not found in seed from perilla mint. Perilla ketone is not only harmful in ruminant but also toxic in small ruminant and mice (Glenn *et al*., 2010).

Perilla mint was used as feed in animals for studying the mechanism of digestion or immunology. The common model species were rats or mice which are easy to manage and observe. Ihara *et al.* (1998) used the oil of safflower and perilla feed in male Wistar rats, 3-weeks old, and observed the lipid metabolism. Two diets either containing safflower oil (SO, high linoleic acid) or perilla oil (PO, high α-linolenic acid) were compared. These diets were fed to rats for 3, 7, 20 and 50 days. The results showed that the level of cholesterol, triglycerides and total lipid in the PO-diet group were lower than in the SO-group feeding the diets for 7 days. This experiment demonstrated that α-linolenic acid plays an important role in the regulation of serum cholesterol and the magnitude of regulation is more powerful when compared to linoleic acid.

Perilla mint has been used to feed animals because of its low costs and abundance throughout East Asia. Oita *et al*. (2008) studied the extraction and digestion of *Perilla frutescens.* In their experiment water-and NaCl-soluble protein fraction contained less trypsin inhibitors than that of soybeans.

As mentioned above, perilla seed and meal are suited as animal feed. Perilla seed was used in polyunsaturated fatty acids (PUFA) enriched diets for growing rabbit aged of 73 days in order to increase the content of n-3 PUFA. Peiretti *et al.* (2010) fed perilla seed to rabbits at a proportion of 10% of the diet. Their results showed that neither digestibility of energy (DE) nor digestibility of protein (DP) and the ratio of DP/DE differed when compared with soybean meal. In during the growth stage of swine fattening, Thirty-one three-way crossbred Landrace×Large White×Duroc pigs, perilla meal was included into diets at 10, 15 and 20% (fresh matter). The 10%-group required the least amount of time to reach the target weight (110 kg) and showed the highest value for daily gain. In contrast, the control group without perilla showed the highest carcass percentages. The 15%-group showed medium back fat thickness. The 20%-group had a thinner back fat thickness of the shoulder than the other groups. Regarding the fatty acid composition of perienal fat, back fat, intermuscular fat and intramuscular fat positive correlations with increasing perilla meal proportions were found. The content of C18:3 increased, while the ratio of n-6/n-3 decreased. Furthermore, the ratio of C18:2/C18:0 and unsaturated fatty acid content also increased in a dose-dependent manner (Yamada *et al.,* 2007). Furthermore, Zang *et al.* (2003) added *Perilla frutescens* in laying hen diets at 8, 12, 16 and 20% (fresh matter), respectively. The results showed that the contents of PUFA increased to 20.42, 23.61, 24.07 and 24.62% in 8, 12, 16 and 20% supplemented groups, respectively, in contrast to 17.03% in the control group ($P>0.05$). The content of omega-3 fatty acid in yolk increased to 6.88, 8.72, 9.86 and 9.95% in the correspondent groups, being significantly higher than in the control group (1.21%, $P<0.01$). There was no significant difference in the total cholesterol (TCH). *Perilla frutescens* could be supplemented in the diet in order to increase the content of PUFA and omega-3 fatty acid, improve the ratio of omega-6 to omega-3 and PUFA to SFA, and decrease TCH and the cost. Likewise, Saito *et al.* (2006) studied feeding perilla oil and seed meal in laying hens and observed α-linolenic acid contents in yolk egg. The α-linolenic acid contents of the egg yolks in perilla oil and perilla meal at the dietary levels of 0, 2.5, 5, 7.5 and 10% each were 0.20, 1.20, 1.64, 2.59 and 3.00 mg/100 mg egg yolk, and 0.17, 0.21, 0.28, 0.32 and 0.39 mg/100 mg egg yolk, respectively. It was suggested that perilla seeds may be the most suitable feed for the production of eggs with high α-linolenic acid content from an economical point of view. Considering eggs are a rich source of dietary cholesterol and cardiovascular disease patient cannot eat egg over one egg per day (Zachary *et al*., 2017). But if we improve fatty acid profile in egg by increase omega-3 it will be a good alternative for cardiovascular disease patient and general person because omega-3 can respect to primary and secondary prevention of coronary heart disease (Clayton *et al*., 2015). So if they eat egg compound with omega-3 maybe reduce the risk cause cardiovascular disease in normal human and patient can eat egg by decrease concern.

Several researches indicated perilla seed and meal can improve fatty acid composition in meat and egg by increasing unsaturated fatty acid, especially omega-3 that has many benefits for human health. Using perilla mint in animal feed is still not pervasive to use in animal feed because the limitation of it such as fiber composition is quite high (problem with monogastric animal), cultivation in some area and harvest once time per year. It is interesting to use for increase value in meat and egg due to the property is high with protein and omega-3.

Conclusion

Seeds of perilla mint, of which all parts of the plant can be used as animal feed, are rich in α-linolenic acid. Through its use as feed, omega-3 fatty acid concentrations in meat and other animal products, i.e., eggs, can be increased, which in turn has positive effects on human health and is known to reduce the risk of different diseases such as coronary artery disease. Additionally, leaves are rich in various bioactive and phenolic compounds and seed meal can be used as protein source. Feeding perilla mint can minimize the contamination with methyl-mercury, which is caused by feeding fish meal. Overall, the use of perilla mint as animal feed has many advantages from a human health perspective and is economically efficient.

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