USING GOLDEN APPLE SNAIL (*Pomacea caniculata*) MEAL AND BETAINE IN LOWLAND FROG (*Rana rugulosa*) FEED

INTRODUCTION

The common lowland frog, kobjarn or kobthong (*Rana rugulosa* Wiegmann), is an economic and interesting amphibian in Thailand because of the high demand for local and foreign market such as Laos, Vietnam, Cambodia, Malaysia, Singapore, Hongkong, Japan, Germany, France and America.. The high price of frog induced the expansion of frog culture in Southeast Asia.

Natural frog is fast growing amphibian when compare to crocodile and snapping turtle. From nursing to market size, it takes 3 – 4 months which similar to other commercial species such as catfish and tilapia. Frog can be raised at all location of Laos and Thailand by using small area and a little quantity of water than other aquaculture species. The frog culture is not success because of high capital but low benefit. Farmers prefer to use commercial feed because of the more comfortable but that cause the high capital. To reduce the feed cost, the farmer can use local feed such as earthworm, snail or home made feed using local material for frog production than commercial feed.

Golden apple snail *(Pomacea caniculata)* meal is one of the local materials available in Southeast Asia that can use as protein source in frog feed. This snail meal is not the good attractant. To improve the palatability, farmer should add some attractant such as synthetic amino acid, fish oil and betaine. Betaine (glycine betaine, trimethylglycine,oxyneurine) is an important feed atttractangt, which is known to stimulate the olfactory bulb of fish. It is found in invertebrates, microorganisms and some plants.

Carnivorous fish and crustaceans often consume invertebrates containing high levels of betaine in the wild. In addition, supplemental betaine to formulated aqua feeds might help to mimic the smell and taste of the diet similar to that of their natural prey organisms. Therefore supplemental betaine in frog feed and using snail meal substitution for fish meal diet could reduce feed cost and increase feed consumption resulting the better performance.

OBJECTIVES

The objectives of this study are as follow:

1. To investigate the appropriate form of frog feed: moist feed and pellet feed.

2. To investigate the attraction effect of betaine in frog feed and effect of betaine on temperature stress tolerance.

3. To investigate the optimal levels of snail meal substituted for fishmeal in frog feed.

LITERATURE REVIEW

General characteristic of Thai native frog, (*Rana rugulosa* Wiegmann)

The local frog has scientific name, *Rana rugulosa.* Common name is lowland frog and Thai name is Kobthong or Kobjarn. Frog in Thailand had 38 species and now the top 5 species preferred in Thai are *Rana Rugulosa, Rana tigerina, Rana marcrodon, Rana blythii* and one foreign species bullfrog (*Rana catesbeiana*). These 5 species are large size and high demand in local and foreign markets. Therefore, farmer interest to culture frog in economically (Chithtaprapong and seetasith, 1985; Department of fisheries, 1993; Lamlirtdesa, 1988; Jilasack, 1992; Boonpran *et al*., 1993).

Habitat and natural

The habitats of lowland frog (*Rana rugulosa*) like that of most frog species, it is usually in or near the water, in damp or wet weather, however, this species frequently wanders for a considerable distance from its aquatic home. It is liable to be found almost anywhere near the shores of lakes, ponds or streams and rivers in the wide territory over which it is distribution. In Thailand, a frog *(Rana rugulosa)* is found from north to south but have many populations in middle part and northeastern part (Vongvichith *et al.*, 2002).

Food and feeding

Lowland frog (*Rana rugulosa*) is a carnivorous amphibian. It's feed consists of earthworm, insects, spiders, worm, small fish and small frog. Duration in adult, frog probably consume insect and small fish such as: tilapia, common carp and silver barb (Namxongxay, 1994). In Southeast Asia, lowland frog (*Rana rugulosa* Wiegmann) was cultured under control condition both in small and large farm. The traditional feed is raw material or the mixture of chopped trash fish and rice bran. This feed often does not completely satisfy the nutritional requirement of frog, resulting in many

malnutrition problems, low growth rate and low survival because frog need feed to obtain a balance their nutritional requirement (Chanpench, 1987; Xengtham, 1999).

In their natural habitat, frogs have variety of foods to select except of times of unfavorable environment or season conditions. Now in Southeast Asia such as Thailand, Indonesia, Malaysia, Singapore and Lao, frog culture for market size usually conduct in concrete tank, floating net cages, in the pond or pond culture. In confinement, frog is removing from their natural source of food to artificial feed, except some of small frog need fed by high protein live food. Therefore, all their nutritional requirements must be supplied to them.

Table 1 Typical growth and feed consumption of bullfrog culture in outdoor pens during 25 to 30°C.

Age in months (post metamorphosis)	Average, weight (g)	Approximate. Daily consumption (g)
0	5	-
1	10	0.50
2	22	1.09
3	35	1.22
4	50	1.72
5	75	2.63
6	105	3.67
7	140	4.90
8	175	6.12

Source: Greg Lutz and Jimmy (1999)

Stocking density

Culture pen or concrete tank size could be 10 – 100 square meter (12 – 120 square yards) in area. After they are taken from tadpole ponds, young frogs can be stocked in culture pen or concrete tank at up 50 frogs per square meter (Greg Lutz and Jimmy, 1999; Boonmanch *et al.*, 1999).

Frog disease

The most common disease of frog, red-leg disease, is due to a bacterial infection *(Aeromonas)*, often resulting from overcrowded conditions. The best preventative methods are adequate nutrition and space. Infective individual should be isolated immediately and treated with antibiotic. In severe cases, it may be necessary to drain the ponds or concrete tanks and allow them to dry out for several weeks (Crawshaw, 1993; Sativalakoon, 1994; Kanchanakhan, 1998; Louis *et al*., 2001).

Nutrient requirements

Rana rugulosa requires the same nutrients as other fish species especially carnivorous species for normal growth and metabolic function. The tadpole of frog can feed by steamed poultry egg soon after the yolk sac absorbed. It can feed by powder feed for catfish, after two weeks and can be fed by small pellet for tadpole or small pellet for catfish. However, a specified amount of a particular nutrient required of frog and catfish are similar. The major nutrients such as protein, and amino acid, lipid and essential fatty acid, carbohydrate, vitamin and mineral are required for normal growth. (Vongvichith *et al*., 2002; Khamsivilay *et al*., 2003),

Protein

Protein is the main essential nutrient for maintaining life and promotion growth. The dietary protein requirements for frog have been determined in various studies. Young tadpoles (1-30days) require protein levels of 38%. Small frogs (30-60 days) require protein levels of 32%. Fattening frog (60-90 days) require protein levels of 26% (Khamsivilay and Phanousith, 2002; Thongkongthay, 2004).

Bullfrog tadpole develops to adult feed by complete feed contained protein 33 % for 4 months; the meat has protein 83 % of dry weight (Thongoulay, 1977). Feeding artificial feed of 35 %, *Rana pipen* tadpole get the best growth rate and frog go to metamorphosis faster than feeding tilapia diet of 23 % protein (Cabre-Pena and polimeni-salinas, 1989). To culture frog with silage fish for 4 months, frog weight reaches to 200g – 400g (Lamlirtdesa, 1988). Using artificial feed of protein 40% mix with silage fish 1:10; feeding rate 3 % of body weight for 4.5 months, the frog grow to 250g (Cithtapraprong and Seetasith, 1985). Using artificial feed of 38 % - 40 % protein and feeding rate of 3% body weight for 4 months, frog weight increase from 10g to 141.03g. Frog (*R. perezi*) when culture with 3 protein levels such as 28%, 39% and 45%, show the same growth rate. The commercially feed of 39 % protein is suitable for culture frog (Martinez *et al*., 1993, Millamena, 1994, Chueaphothihack, 1999, Millamena *et al.*, 2002).

Frog meat is a white meat with high nutritional value. It has ten amino acids. It is almost fat free, without cholesterol and with a very high digestibility. Due to these excellent nutritional qualities, frog meat is recommended in several therapy diets.

Table 2 Composition of frog meat and other meat composition

Species	Calories (Kcal)	Protein (g)	Fat (g)
Frog	68	16.4	0.3
Chicken	264	18.1	18.7
Beef	225	19.4	15.8
Pig	276	16.7	22.7
Rabbit	162	21	8.0

Source: Favier, (1999)

Fat and fatty acid

Frog is a fast growing animal that need high energy and high quality fat especially the essential fatty acids because the body must have them to survive, but cannot synthesize them from any other substance. Only two kinds of essential fatty acids *omega 3 (n-3 or w3) and omega 6 (n-6 or w6)*, both of which are unsaturated fatty acids. Should provide enough in the right ratio. Each EFA is turned into several derivatives by the body and use in the metabolism. Omega 9 (monounsaturated), omega 7, and saturated fat, are non-essential because the body can produce them from sugars and starches.

Sources of n-3s are flaxseeds and green leaf vegetables. The n-3 *derivatives* EPA and DHA are found in high fat, cold water fish such as albacore tuna, sardines, Atlantic halibut and salmon, coho, pink and king salmon, Pacific and Atlantic herring, Atlantic mackerel, and lake trout. Small amounts of EPA and DHA n-3s are also found in oysters and other shellfish. Omega 6 is found in sesame and sunflower seeds and other seeds and nuts. Land animal meats and fish flesh are sources of the Omega 6

derivative, arachidonic acid (AA). The fish listed above are preferred sources of n-3 and n-6 derivatives, because they are the richest sources

Fatty acid composition of bullfrog oil after heat at different times is presented in Table 3

Table 3 Fatty acid compositions of bullfrog oil

Fatty acid Percentage by weight	Time (minutes)				
	0	5	15	30	60
14:0	1.9	2.8	2.7	2.7	2.7
15:0	0.5	0.6	0.5	0.51	0.5
16:0	17.5	17.6	18.1	8.0	18.1
18:0	5.1	3.9	4.1	4.1	4.1
16: l n-7	7.8	8.5	8.0	8.23	8.0
18: l n-9	43.2	35.9	34.7	4.3	31.7
20: l n-9	1.3	1.2	1.3	1.21	1.3
18: 2 n-6	8.1	12.6	12.9	2.7	12.9
18: 3 n-3	0.9	1.3	1.4	1.4	1.4
20: 5 n-3	1.0	1.3	1.5	1.5	1.5
22: 5 n-3	0.6	0.9	1.0	1.0	1.0
22: 6 n-3	3.6	3.7	4.7	4.52	4.7
SAT	25.0	24.9	25.4	5.3	27.4
MUFA	52.3	45.6	44.0	43.7	46.7
PUFA	14.2	19.8	21.5	21.1	24.3
TOTAL	91.5	90.3	90.9	90.1	87.9

Note: SAT, saturated fatty acids;

MUFA, monounsaturated fatty acids

PUFA, polyunsaturated fatty acids. Includes fatty acids in percentages grater than 0.5%.

Source: Méndez *et al* (1998)

Carbohydrate

The nutritional value of various form of dietary carbohydrate is one of the least understood aspects of frog nutrition. Fresh and warm-water fish, including catfish, seabass can use much higher levels dietary digestible carbohydrate than cold-water or marine fish (Wilson, 1980; NRC, 1993). Although no dietary requirement for carbohydrate has been demonstrated in fish, but it is important to provide the appropriate amount of digestible carbohydrate in fish diets because carbohydrate are the lease expensive energy source. Enzymes for digestion and metabolism of carbohydrate have been detected in several fish species. Wilson (1985) observed that channel catfish fed a high carbohydrate diet stimulated several lipogenic enzyme activities in both liver and adipose tissue.

A typical commercial catfish diet contains 25 to 30% carbohydrates. These compose of 3-6% indigestible carbohydrate for catfish and carnivorous species generally present as crude fiber. Thus it is not desirable in the diet because indigestible material may “pollute” the water. Carbohydrates are useful not only a source of energy but also a binder because of their viscosity. Carbohydrates will hold together ingredients and reduce the rate at which a feed will dissolve in water

Snail meal

The African giant snail (*Achatina fulica*) originate from Africa, is now widespread throughout the entire Southeast Asia and the Pacific. Some snails are quite large. African giant snail can be over 30 cm long. Snails are a viable supplemental protein source in some parts of the world and can be used to replace other animal Protein sources in the rations. Freshly collected snails are broiled in water for 15 to 20 minutes. The flesh is separated from the shell, minced and dried at a temperature of more than 60 C. The adult *Helix aspersamaxima*has live weight is about 40g. The shell portion is around 15% of live weight. Feeding raw snails give poor growth (Creswell, 1981). Boiling snails for 10 – 20 minutes prior to feeding or drying is improving animal performance (Creswell and Habibie, 1981). Supplementation with 0.2% methionine in a diet containing 20% snail meal further improved growth in poultry (Creswell and Habibie, 1981).

Snail meal when fed at 4, 8, 12 % levels in the diets of broilers is found to be a suitable replacement for fishmeal and meat and bone meals (June, 1991). Fifty percent replacement of fishmeal with snail meal in broiler diets showed no depression in growth or feed conversion. Snail fed up to 5 % of the diet does not depress egg weight and up to 10 % do not depress egg production in layers (Creswell, 1981). Up to 15 % golden snail meal is found to be able to be fed to layers without depressing performance. Snail meal is found to be comparable to fish meal when used supplementation protein source in layer diets. Ducks can tolerate up to 50% snail meal. Tilapia performed best when 75% snail meal + 25% rice bran is fed. Snail meal is found to have a slightly lower value than fish meal in diets for tilapia (Caguan and Doria, 1989, Caguan and Joshi, 2002).

The composition and the essential amino acid profile of snail mealare present in table 4 and 5.

Table 4 Chemical composition of snail meal (% in dry matter)

Parameter	Snail meal	Snail meal	Snail meal	Snail meal *(Helix sp)*	African giant snail
DM	-	-	89.7	94.3	88.9
CP	53.9	60.9	50.6	50.6	51.3
CF	-	4.5	-	-	-
Ash	3.6	9.6	19.9	19.9	7.9
EE	-	6.1	8.6	8.6	2.7
NEF	4.78	18.9	10.6	10.6	-
Ca	0.85	2	-	-	0.83
P	-	0.48	-	-	0.54

Note: DM: Dry meter
CP: Crude protein
CF: Crude fiber
EE: Ether extract
NFE: Carbohydrate
Ash: Ash
Ca: calcium
P: Phosphorus

Source: Caguan and Doria (1989)

Table 5 Essential amino acid profile of snail meal (g/16gN)

Essential amino acid	Percentage of protein
Arginine	4.88
Histidine	1.43
Isoleucine	2.64
Leucine	4.62
Lysine	4.35
Methionine	0.89
Phenyalanine	2.62
Threonine	2.76
Tryptophan	-
Valine	3.07

Source: Hertrampt and Piedad-Pascual (2000)

Disease snail

Snail is potential human health risk, involved in transmitting meningitis to people when eaten uncooked and a serious pest of rice, banana and other vegetable crops. The common fields slug (*Agriolimax meticulatus*), a shells snail, is an intermediate host of the sheep lungworm. The golden apple snail (*Pomacea caniculata*) is also a pest of rice fields and *Cerithium tenellum* and *Telescopium telescopium* are pests in milkfish ponds (Hertrampt and Piedad-Pascual, 2000)

Robison, (2004) reported that snail damage to plant in tropical and subtropical agricultural systems and environment. These snails are also known to carry organism that can cause serious disease in humans, including *Angiostrongylus cantonensis* and

potentially *A. costaricensis.* These organisms can be transferred by ingesting improperly cooked snail meat or by handling live snail and allowing their mucus to contact human mucous membranes such as those in eyes, nose and mouth (World health reported, 2002).

Muirson (2004) said that the life cycle of the liver fluke, a flat leaf-like parasite of mammals, has aquatic snail to be the intermediate host. How flukes cause damage, in freshwater when intermediate snail hosts release infective forms of the parasite. People are infected by contact with water where infected snails live.

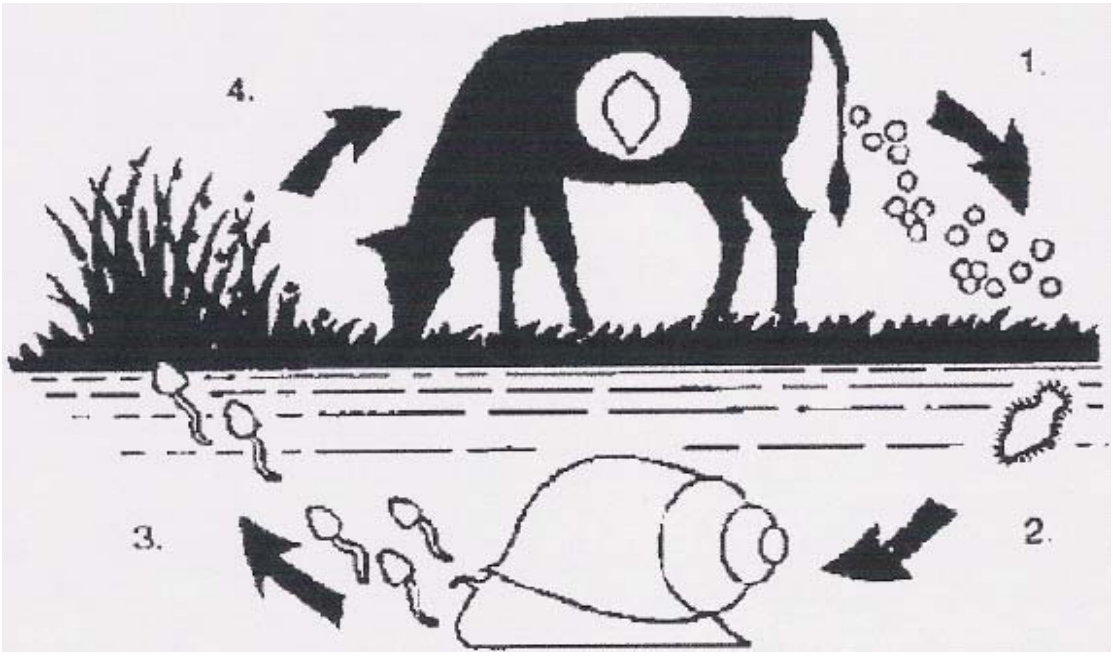


Figure 1 The life cycle of the fluke

For treatment and prevention, the large number of anthelminthic is available for treating fluke in cattle. As fluke tends to be chronic, most will be effective, but seek veterinary. Where is present fluke including cattle from typical snail habitats (low lying areas margin of pond) can reduce fluke infection but complete snail avoidance is impossible as it is to difficult to identify all snail site. Drainage to eliminate the sail offers the effective means of control indeed. Since 1970, chemical control has been used to reduce snail number but is no longer available (Richard, 2003).

Betaine

Source betaine

Betaine is a natural occurring product in many plants and animals species. Betaine in relatively large quantity in sugar beets and aquatic invertebrates but is not present in significant quantity in most animal foodstuffs. Chemically betaine is trimethylglycine and the major involvement of betaine in lipids metabolism is in its lipotropic activity. Food sources of betaine include beets, liver, eggs, fish, legumes and whole grains. High level of betaine is found in many of the natural prey animals of fish, especially in marine invertebrates. However the main foodstuffs for aquaculture diets contain little if any betaine. Hence the betaine intake of farmed fish is generally much lower than that of their wild counterparts (Briefing, 1996). Betaine HCl is also commonly used as a nutritional supplement to increase gastric acidity. The betaine in this compound dose not alters gastric acidity but simply delivers the hydrocholric acid (Alternative; 2003).

Function betaine

The chemical structure of betaine (glycine betaine, tri-methylglycine) is shown in figure 2. Betaine has two major metabolic functions, as an osmoprotectant and as methyl donor. In addition, betaine is an attractant for many aquatic species. These properties have been widely used by animal feed industry to improve animal performance, especially under sub optimal conditions.

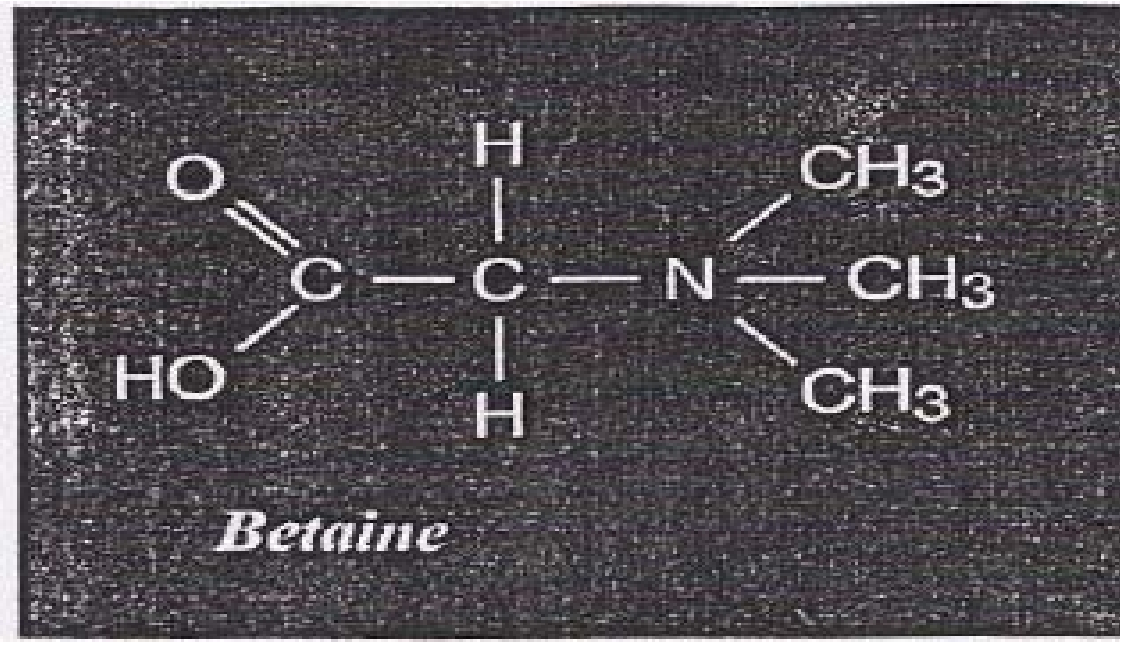


Figure 2 The chemical structure of betaine

Betaine is a non-toxic natural physiological compound, also used in foodstuffs and pharmaceuticals. It tolerates temperature up to 200°C and hence is stable in feed processing, including extrusion.

Betaine as osmoprotectant

The main reason why betaine is found in high quantity in some plant microbial and animal species is the ability to protect cells against osmotic inactivation. In these case, exposure to drought or high salinity triggers betain snythesis in mitochondria, resulting in betaine accumulation in the cells. Betaine being a compatible osmolyte increases the water retention of cells replaces inorganic salt and protects intracelluar enzyme against osmotically or temperature-induced inactivation. A similar function of betaine has been demonstrated in mammalian kidney; however, betaine accumulation in this organ appears to be mainly from exogenous origin rather than a result of *de novo* synthesis (Briefing**,** 1996; Alternative, 2003).

While some organisms can increase their betaine synthesis during osmotic stress, many are completely or partially dependent on an exogenous source of betaine for osmotic protection. For example, isolated salmon liver mitochondria exposed to osmotic stress showed a reduction in betaine synthesis, while uptake of betaine into the mitochondria was strongly stimulated. Osmotially stressed mitochondria showed much less respiratory inactivation when betaine was in the incubation medium. It is obvious supplemental dietary betaine results in betaine accumulation in the tissue of osmotically stressed fish where it helps the cells to maintain their ionic and osmotic balance and metabolic activity (Briefing**,** 1996).

Betaine as methyl donor

Betaine donates its methyl groups in several enzyme-catalyzed reactions essential for the protein and energy metabolism of fish and shrimp. These include the synthesis of methionine from homocysteine and the syntheses of carnitine and phosphatidyl choline. While some betaine can be synthesized through oxidation of choline in mitochondria, the efficacy of this conversion is often not high enough to meet the betaine requirement for its methylation function, or especially for its osmoprotectant function (Briefing**.** 1996). The relationships between choline, betaine and methionine.

Betaine as attractant

Betaine as mentioned above many aquatic invertebrates contain significant amount of betaine. Hence it is not surprising that many fish species are receptive to betaine, a major and highly water soluble compound of their prey animal, as a smell and taste attractant. The attractant effect of betaine has been demonstrated in salmonids and shrimp (Briefing**,** 1996).

MATERIALS AND METHODS

The study of using snail meal and betaine in lowland frog (*Rana rugulosa*) feed was divided into two experiments. The first experiment was investigated on appropriate form of frog feed and supplemental betaine in frog feed by focusing on moist feed and pelleted feed supplemental betaine 1.5% in diet. Frog with size of 4 – 6 cm and 10 – 15 g body weight were stock at the rate of 50 frogs per tank. Four experimental diets were formulated, 0% betaine in moist feed, 0% betaine in pellet feed, 1.5% betaine in moist feed and 1.5% betaine in pelleted feed. The diet was isonitrogenous (40±1% CP) and is calories (3,100±100 DE kcal/kg). The feed formula was shown in table 6. Second experiment was studied on replacement fishmeal with golden apple snail meal in frog diets by varying percentage of snail meal substituted for protein from fish meal at the levels of 0, 50, 100 % (0, 22.5 and 45% by weight) and 0 % + 1.5% betaine. Four diets were formulated from practical ingredients and feed formula was shown in table 8 isonitrogenous (43±1% CP) and isocalories (3,100±100 DE kcal/kg). Feed composition was shown in table 9. This experiment was investigated in two frog size. Trial 2.1, young frog with size of 20 – 30 g and trial 2.2, grower frog with size of 60 – 75g were studied.

Materials

Experimental diet

Experimental diet 1

The diet formula for experimental 1 was presented in table 6. Diet was prepared to two forms, moist and pellet feed each of them supplemental with betaine 0 and 1.5%. The carbohydrates was streamed and mix with fine ground raw material after that added 15% water and pellet by pass through mincer with diameter of 2.0 mm. The diets were isonitrogenous (40 ±1%CP) and isocalorics (3,100±100DE kcal/kg). Feed formula was shown in Table 6.

Table 6 Composition of frog feed for experiment 1

Raw material	Composition ingredient	
	0 % Betaine	1.5 % Betaine
Fishmeal (%)	40	40
Soy bean meal (%)	20	20
Rice bran (%)	20	20
Wheat flour (%)	15	13.5
Vitamin& mineral (%)	2	2
Vegetable & fish oil (%)	3	3
Betaine (%)	-	1.5
Total	100	100

Note: [1] Vitamin and mineral (mg/1,000g of feed): Vitamin C with Vitamin A 4,000IU, Vitamin D_3 2,000 IU, Vitamin E 50 mg, Vitamin K 10 mg, Thiamin 20mg, Riboflavin 20 mg, Pyridoxine 20mg, Calcium pantothenate 200mg, Niacin 150 mg, Biotin 2.0 mg, Folic acid 5mg, Vitamin B_{12} 0.2 mg, Inositol 400 mg, Ethoxyquin 200 mg and mineral composed with Iron 30 mg, Zinc 20 mg, Magnesium 25 mg, Copper 5 mg, Cobalt 0.05 mg, Iodine 5 mg and Selenium 0.2 mg.

The proximate composition of ingredients such as: moisture, protein, lipid, fiber, ash and NFE (nitrogen free extract) of raw material before preparing the artificial feed were analyzed by the following:

- Moisture was determined by drying sample in an oven at 80 ^{0}C- 105 ^{0}C for 24 hours (AOAC, 2000)
- Crude protein was determined indirectly by measuring the total nitrogen by the standard Kjedahl method modifies from Yoshida multoplied by an empirical factor of 6.25. The Titrimetric determination will be used for nitrogen determination (AOAC, 2000).
- Crude lipid was determined by using the Soxtec system Buchi 800 with using petroleum ether (AOAC, 2000).
- Crude fiber was determined by the acid-base digestion method using Fibertec (AOAC, 2000).
- Ash content was determined by burning the samples in muffle furnace at 600 ^{0}C for 2 hours (AOAC, 2000).

Table 7 The proximate composition of ingredients used in formulated experimental 1 diets (as fed)

Ingredients	Fishmeal	Snail meal	Soy bean meal	Rice bran	Wheat four
Moisture (%)	7	6.4	9.6	9	11
Protein (%)	60	52	43	12	10.8
Lipid (%)	9.05	2.5	2.3	15	0.9
Fiber (%)	4	8.5	4.7	6.2	0.5
Calcium (%)	5.8	4.5	7.4	0.06	-
Phos (%)	0.13	0.02	0.04	0.47	-
Ash (%)	15.6	23.9	6.9	8.1	0.5
NFE	4.35	6.7	33.5	49.7	70

Table 8 The proximate composition of experimental diets containing 0 and 1.5% betaine in frog feed

Raw material	Commercial frog feed	
	0 % Betaine	1.5 % Betaine
Moisture (%)	8.42	8.43
Ash (%)	13.2	14.00
Crude protein (%)	40.83	39.97
Fat (%)	9.05	8.77
Fiber (%)	2.72	3.78
DE kcal/kg	3,001	2,926
NFE (%)	25.78	25.05

Experimental diet 2

The diet formulas for experiment 2 was shown in table 9 snail meal was substituted for fish meal at the level of 0, 50, 100% and 0 % +1.5% betaine. Feed forms pellet feed and betaine level was designed by follow experiment 1 results.

Table 9 Composition of frog diets using snail meal substituted for fishmeal and supplemental 1.5% betaine

Material	Snail substituted for fishmeal in frog diet			
	0 %	50%	100%	0%+1.5% betaine
Fishmeal	40	20	0	40
Snail meal	-	22.5	45	-
Soy bean meal	20	20	20	20
Rice bran	20	20	20	20
Wheat flour	15	7	1.5	13.5
Vitamin & mineral	2	2	2	2
Vegetable & fish oil	3	5	6.5	3
Betaine	-		-	1.5
Di-calcium	-	3.5	5	-
Total	100	100	100	100

Table 10 The proximate composition of experimental diets containing snail meal substituted for fish meal in frog feed

Composition	Snail meal substituted for fish meal in frog diets			
	0 %	50 %	100 %	0 %+1.5% betaine
Moisture (%)	9.4	9.6	9.5	9.4
Ash (%)	14.2	16.0	14.79	13.58
Crude protein (%)	43.8	43.93	43.75	43.87
Fat (%)	11.3	10.67	10.47	10.30
Fiber (%)	2.39	3.28	3.32	3.15
DE kcal/kg	3,128	3,023	3,041	3,071
NFE (%)	18.91	16.52	18.17	19.7

Experimental set-up

The study of using snail meal and betaine in lowland frog (*Rana rugulosa*) diet was divided into two trails.

Experiment 1: Study on appropriate from and attraction effect and temperature stress tolerance effect

1.1. Studies on attractant effect

One frog was stocked in each of 80 L aquarium, which was separated by plastic net to two parts, left and right side. The left side use for stock frog and the right side use for put moist feed and pellet feed of 20g on the tray. After put feed on the tray, open plastic net, record the weight of feed consume every day for 3 days.

1.2. Study on appropriate form of feed

Small frogs of 50 g were stocked individual in $1m^2$ tanks with water level of 5-10 cm and fed with experimental diet of 5 % of body weight by separate to two times a day. One hour after feed, the uneaten feed was collected and weigh for calculating feed consumes the culture tank was cleaned and changed water every 3 days. Frog was raised 1 month. Feeds consume and growth performance was study at the end of 1 month research.

1.3. Study on temperature stress tolerance and blood osmolarity

Frog fed with betaine supplemental diet, pellet form, for one month was stocked in temperature control chamber at 40 $^{\circ}$C, 27 $^{\circ}$C and 15 $^{\circ}$C for 6 hours after that sampling blood for determine Na^+, K^+, Cl^-, osmolanity, red blood cells, hemoglobin, hematocrit. To evaluate the temperature stress tolerance (osmoprotectant effect) of betaine.

Experiment 2: Study on optimal level of substitution snail meal for fish meal in frog diet.

This study was investigated in completely randomized design (CRD) with four treatments and three replicates during culture period 28 days. The treatments were artificial pellet feed contained snail meal 0, 50, 100 % and 0% mixed 1.5% betaine substituted for fish meal. The feed formula was shown in table 9. This experiment was divided into two trails 2.1, study on young frog and trial 2.2, and study on grower frog.

Experimental condition

Trail 2.1: young frog *(Rana rugulosa)* with average size of 6 - 8 cm and 20 - 30g body weight was stocked 12 individual per $1m^2$ tanks at water level of 5-10 cm and fed with experimental diet of 5 % body weight by separate to two times, 07:00 – 08:00 am and 16:00 – 17:00 pm. One hour after feed, the uneaten feed was collected and weight for calculated feed consume. The culture tank was cleaned and changes water every 3 days. The study period culture was 1 month.

Trail 2.2: young frog *(Rana rugulosa)* with average size of 8 - 10 cm and 60 - 70g body weight was stocked 15 individual per $1m^2$ tanks at water level of 5-10 cm and fed with experimental diet of 5 % body weight by separate to two times, 07:00 – 08:00 am and 16:00 – 17:00 pm. One hour after feed, the uneaten feed was collected and weight for calculated feed consume. The culture tank was cleaned and changes water every 3 days. The study period culture was 1 month

Both of trail 2.1 and 2.2 were 0f using growth performance and feed efficiency. Only trails 2.2 investigated hematological values and blood osmolarity. The water quality also monitored.

Figure 3 Moist and pellet feed for (experiment 1)

Figure 4 Snail meal substituted for fishmeal for (experiment 2)

Figure 5 Experimented at concrete tank showing indoor lab

Figure 6 Moist feed for (experiment 1)

Data collections and analytical methods

1. Frog growth performances

Growth performance such as average daily weight gain, percentage of weigh gain and specific growth rate was studied. Frog fed with experimental diets containing 0, 50, 100 and 0 % +1.5% betaine was evaluated 2 weeks /time until the end of study. The following indices were used to evaluate the frog growth:

- **Average daily mean weight gain ADG (g/day)**

$$= \frac{\text{Mean final frog weight} - \text{Mean initial frog weight}}{\text{Culture period (days)}}$$

- **Percentage of weight gain (%)**

$$= \frac{\text{Mean final frog weight} - \text{Mean initial frog weight}}{\text{Mean initial frog weight}} \times 100$$

- **Specific growth rate, SGR (%)**

$$= \frac{\text{Ln final frog weight} - \text{Ln initial frog weight}}{\text{Culture period (days)}} \times 100$$

- **Survival rate (%)**

$$= \frac{\text{Final Number of frog}}{\text{Initial number of frog}} \times 100$$

2. Feed efficiency

The study on feed efficiency such as feed consume, feed conversion ratio (FCR) protein efficiency ratio (PER) in frog feed with snail meal substituted for fish meal diets were determined at the end of the experiment. The following indices were used to evaluate the feed efficiency:

- **Feed intake (g/frog/day)**

$$= \frac{\text{Feed intake daily study period (g)}}{\text{period of study (day)}}$$

- **Feed consumed (%)**

$$= \frac{\text{Feed consume weight x 100}}{\text{Frog body weight (g)}}$$

- **Feed conversion ratio (FCR)**

$$= \frac{\text{Total feed intake (g)}}{\text{Mean weight gain (wet weight basis) (g)}}$$

- **Protein efficiency ratio (PER)**

$$= \frac{\text{Wet weight gain (g)}}{\text{Dry protein intake (g)}}$$

- **Net protein utilization (NPU)**

$$= \frac{\text{Protein in final frog body – Protein in initial frog (dry weight)}}{\text{Protein intake (dry weight)}}$$

3. Hematology study

Three frog from each tank were collected 0.5 – 1.0 ml blood from heart and prevent blood cloth by EDTA from determined red blood cell, hematocrit and hemoglobin determined by automatically blood cell count machine (Sysmex F. 820) follow the method of Sirois (1995).

4. Temperature stress tolerance and blood osmolarity

Frog in each treatment was divided into three groups and stocked in temperature control chamber at 40°C, 27 °C and 15 °C for 6 hours after collected heparin blood for determined Na^+, K^+, Cl^- by (Electrolyze and lyzer Model 644 Ciba corning) and osmolarity by automatically machine (Osmometer Model 3D3 Advanced Instruments).

5. Water quality analysis

a. Temperature was measured air temperature and water temperature directly in the water of tank two times every day 8:30 – 16:30 h (minimum and maximum) by using thermometer.

b. Water quality pH, DO, total ammonia (NH_3), biochemical oxygen demand (BOD), nitrite (NO_2), orthophosphate (OP) and total phosphate (TP) was measured follow APHA- AWWA (1995) before change water and after change water of tank 2 weeks/time.

6. Statistical analysis

All data were analyzed by one-way ANOVA (analysis of variance). The Duncan's Multiple Range Test was used to determine the differences between the treatment means. The alphabetical notation was used to mark the differences at significant level of an Alpha 0.05 (Gomeeze and Gomeze, 1984).

7. Place and duration of study

The experiment was carried out for treatment diets of frog in the concrete tank set up indoor laboratory of Department of Aquaculture, Faculty of Fisheries, Kasetsart University, started from July to December 2005. Proximate analysis of raw materials, artificial feed and frog was conducted in fish nutrition laboratory, Faculty of Fisheries, Kasetsart University Fish Feed Technology Development Center.

RESULTS

The study of using golden apple snail meal and betaine in lowland frog feed was divided into two experiments. Experiment 1: study on appropriate form of frog feed and supplemental betaine in frog feed. Experiment 2: to investigate the suitable levels of snail meal substituted for fishmeal in frog feed.

Result of experiment I

Study on appropriate form of frog feed and supplemental betaine in frog feed by using moist feed and pellet feed and supplemental 0 and 1.5% betaine in diet was investigated. The results of this research as follow:

Attraction effect

The Appropriate form of frog feed was determined by amount of feed consumed in table 11.

Table 11 Feed consume frog fed moist and pellet feed

Parameter	Form of frog feed					
	Moist feed		Pellet feed		P.value	Pool.SE
	0 %	1.5 %	0 %	1.5 %		
Feed consume g/day/frog	2.49 [a] ± (1.34)	3.08 [a] ± (1.61)	2.63 [a] ± (1.37)	3.84 [a] ± (1.36)	0.6367	1.4313

Note: [a, b] and [c] in the same row having the same superscript were not significantly different (P>0.05).

Feed consume of frog fed with moist and pellet feed supplemental with 0 and 1.5 % betaine were not significantly different (P>0.05).Although, feed composed with 1.5% betaine showed a little bit higher feed consume than 0 % betaine feed.

Growth performance of frog

The growth performances of frog in different experimental treatment were evaluated and shown in table 12 and fig. 7. Mean initial weight of all experimental frog were not significantly differences (P>0.05), Mean final weight increased the higher in group of frog fed pelleted feed both 0 and 1.5% betaine (P<0.05).

Mean frog weight gain were increased in all experimental treatment with form of feed. Mean weight gain of frog fed moist feed 0% betaine (18.28 g) and moist feed 1.5% betaine (22.56 g) did not show significantly differences (P>0.05). for pelleted feed 0% (34.29 g) and pellet feed 1.5% betaine (34.25 g) betaine also did not show significantly differences (P>0.05). But when compare the growth performance of frog fed moist feed and pelleted feed, there were significantly different (P<0.05). Pelleted feed showed better growth performance.

Table 12 Growth performance of frog fed pellet and moist feed during 28 days (mean ± SE)

Growth performance	Frog feed				P.value	Pool.SE
	Moist feed		Pellet feed			
	0 %	1.5 %	0 %	1.5 %		
Mean initial Weight(g)	12.58 [a] ± (0.23)	12.40 [a] ± (0.13)	12.63 [a] ± (0.11)	12.76 [a] ± (0.17)	0.1483	0.1713
Mean final Weight(g)	30.86 [a] ± (3.24)	34.96 [a] ± (5.12)	46.93 [b] ± (6.19)	47.02 [b] ± (1.88)	0.0038	4.4357
Mean weight Gain (g)	18.28 [a] ± (3.47)	22.56 [a] ± (5.11)	34.29 [b] ± (6.11)	34.25 [b] ± (1.83)	0.0042	4.4456
Percent of weight gain (%)	145.64 [a] ± (30.16)	181.94 [a] ± (41.48)	271.28 [b] ± (46.69)	268.30 [b] ± (14.08)	0.0052	35.3891
Average Daily weight gain (g)	0.65 [a] ± (0.12)	0.80 [a] ± (0.18)	1.22 [b] ± (0.21)	1.22 [b] ± (0.06)	0.0043	0.1600
Specific growth rate(%/day)	3.19 [a] ± (0.44)	3.67 [a] ± (0.54)	4.66 [b] ± (0.43)	4.65 [b] ± (0.13)	0.0056	0.4190
Survival rate (%)	80 [a] ± (0.57)	88 [a] ± (2.64)	86.66 [a] ± (2.40)	81.33 [a] ± (2.33)	0.5136	7.4610

Note: [a,b] and [c] in the same row having the same superscript were not significantly different (P>0.05).

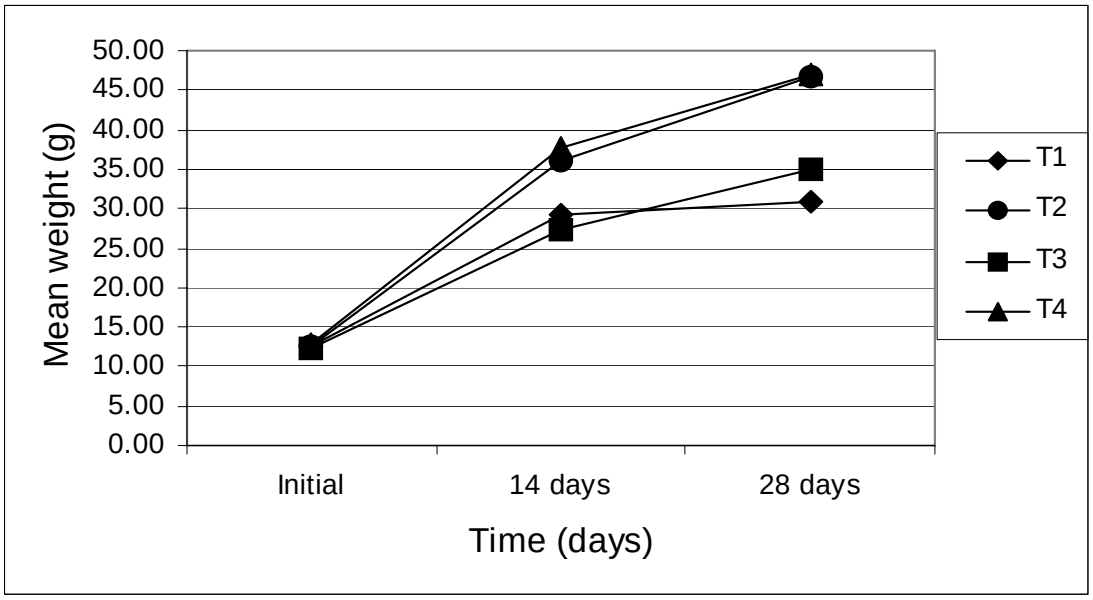


Figure 7 Growth of frog fed with different form of feed during 28 days

Note: T_1 = Moist feed 0 %

T_2 = Pellet feed 0 %

T_3 = Moist feed mixed betaine 1.5 %

T_4 = Pellet feed mixed betain 1.5 %

Percentage of weight gain (PWG), average daily weight gain (ADG) and specific growth rate (SGR) were significant differences ($P<0.05$) among all treatments. Frog fed pellet feed containing 1.5% betaine showed the high ($P<0.05$) PWG, ADG and SGR than frog fed moist feed containing 0 and 1.5% betaine.

Percentage of weight gain (PWG) in frog fed moist feed containing 0 and 1.5% betaine and pellet feed of 0 and 1.5% betaine were significantly different ($P<0.05$). The results in table 12 indicated that PWG in group of pelleted feed was higher (271.28%) than group of moist feed (14564%) ($P<0.05$). There were not significantly different ($P>0.05$) between frog fed diets containing 0 and 1.5% betaine in both form. The percentage of weight gain decreased by form of feed. Average daily weight gain (ADG) in frog fed moist feed containing 0 and 1.5% betaine and pellet feed of 0 and 1.5% betaine were significantly different ($P<0.05$). The results in table 12 indicated that ADG in group of pellet feed was higher (1.22g) than group of moist

feed (0.65g) ($P<0.05$). There were not significantly different ($P>0.05$) between frog fed diets containing 0 and 1.5% betaine in both form. The Average daily weight gain decreased by form of feed.

Specific growth rate (SGR) in frog fed moist feed containing 0 and 1.5% betaine and pelleted feed of 0 and 1.5% betaine were significantly different ($P<0.05$). The results in table 12 indicated that SGR in group of pelleted feed was higher (4.65 %) than group of moist feed (3.19 %) ($P<0.05$). There were not significantly different ($P>0.05$) between frog fed diets containing 0 and 1.5% betaine in both form. The Specific growth rate decreased by form of feed.

Survival rate of frog fed with different diets showed not significantly different ($P>0.05$). The survival rate ranged from 80.00 – 88.00%. survival rate of frog fed different diets was presented table12 and figure 8.

Table 13 Survival rate of frog fed different form of diets during 28 days

Time (days)	Frog feed			
	Moist feed		Pellet feed	
	0% Betaine	1.5% Betaine	0% Betaine	1.5% Betaine
0	100.00	100.00	100.00	100.00
14	100.00	100.00	100.00	100.00
28	80.00	88.00	86.66	81.33

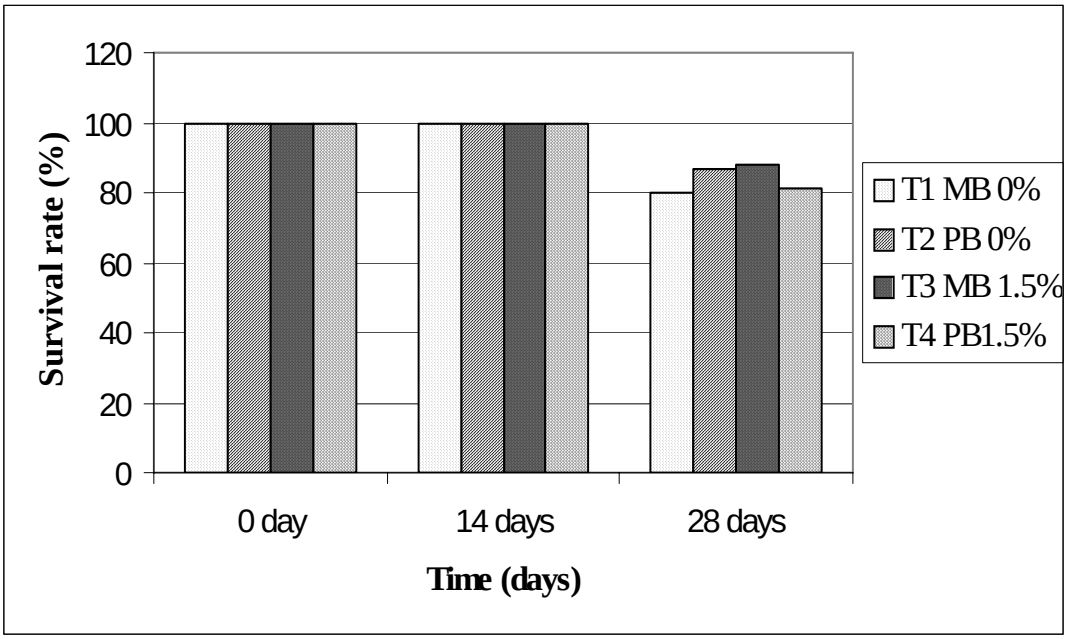


Figure 8 Survival rate of frog fed different form of diets during 28 days

Note: MB: Moist Betaine

PB: Pellet Betaine

Feed efficiency of the experimental frog

Feed intake (FI) of frog fed moist feed containing 0 and 1.5% betaine and pelleted feed of 0 and 1.5% betaine were significantly different ($P<0.05$). The results in table 14 indicated that FI in group of pellet feed was higher (1.36 – 1.49 g) than group of moist feed (0.89 -1.06 g) ($P<0.05$). There were not significantly different ($P>0.05$) between frog fed diets containing 0 and 1.5% betaine in both form. The Feed intake decreased by form of feed.

Table 14 Feed efficiency of frog fed different form of diets during 28 days (means ± SE)

Feed efficiency	Frog feed					
	Moist feed		Pellet feed		P.value	Pool.SE
	0 %	1.5 %	0 %	1.5 %		
Feed intake (g/frog/day)	1.06 [a] ± (0.02)	0.89 [a] ± (0.08)	1.36 [b] ± (0.10)	1.49 [b] ± (0.14)	0.0078	0.1673
Percent feed intake (%)	1.23 [a] ± (0.06)	096 [a] ± (0.15)	1.17 [a] ± (0.13)	1.24 [a] ± (0.09)	0.3504	0.2037
Feed conversion ratio FCR	1.67 [a] ± (0.09)	1.17 [a] ± (0.07)	1.16 [a] ± (0.04)	1.21 [a] ± (0.02)	0.2671	0.3390
Protein efficiency ratio (%)	0.45 [a] ± (0.04)	0.56 [a] ± (0.08)	0.84 [b] ± (0.07)	0.86 [b] ± (0.02)	0.0042	0.1106

Note: [a,b] and [c] in the same row having the same superscript are not significantly different (P>0.05)

The percentage of feed intake (PF) in frog fed moist feed containing 0 and 1.5% betaine and pelleted feed of 0 and 1.5% betaine were not significantly different (P>0.05). The results in table 14 indicated that PF in all groups was not significantly different (P>0.05). The value range from 0.96 – 1.24 %.

Feed conversion ratio (FCR) in frog fed moist feed containing 0 and 1.5% betaine and pellet feed of 0 and 1.5% betaine were not significantly different (P>0.05). The results in table 14 indicated that FCR in all groups was not significantly different (P>0.05). the value range from 1.16 – 1.67.

The protein efficiency ratio (PER) in frog fed moist feed containing 0 and 1.5% betaine and pellet feed of 0 and 1.5% betaine were significantly different ($P<0.05$). The results in table 14 indicated that PWG in group of pellet feed was higher (0.84 %) than group of moist feed (0.45 %) ($P<0.05$). There were not significantly different ($P>0.05$) between frog fed diets containing 0 and 1.5% betaine in both form. The protein efficiency ratio (PER) decreased by form of feed

Hematology and blood osmolarity

The blood compositions of experimental frog fed different betaine level were collected at the end of one month study. The values were presented in table 15. The parameter such as osmolarity, red blood cell, hematocrit, hemoglobin, Na^+, K^+, Cl^- and pH were measured

The osmolarity was observed from frog fed diets containing 0 and 1.5 % betaine which incubated at different temperature for 6 hours. The high temperature (40^OC), ambient temperature ($27\ ^OC$) and low temperature ($15\ ^OC$) showed no significantly difference ($P>0.05$) between these betaine levels. Osmolarity of frog fed 0 and 1.5% betaine at high temperature were 251.25 and 235.75 mmol/kgH_2O, respectively. Osmolarity at ambient temperature were 237.25 and 233.25 mmol/kgH_2O, respectively. Osmolarity at low temperature were 222.00 and 218.50 mmol/kgH_2O, respectively. Osmolarity at low temperature showed significantly difference ($P<0.05$) with high and ambient temperature in both supplemental betaine and without betaine treatment.

The red blood cells (RBC) and hematocrite(Hct) showed no significantly difference ($P>0.05$). RBC of frog fed 0% betaine at high temperature, ambient temperature and low temperature were 0.17, 0.18, 0.26 $x10^6$ cell/ml, respectively. RBC of frog fed 1.5% betaine were 0.19, 0.19 and 0.20 $x10^6$ cell/ml, respectively. The hematocrite (Hct) observed from frog fed diets containing 0% betaine at high temperature, ambient temperature and low temperature were 2.92, 2.62 and 5.35%, respectively. Hct of frog fed 1.5% betaine were 3.13, 2.90 and 3.55%, respectively.

The hemoglobin (Hg) observed from frog fed diets containing 0 and 1.5% betaine showed no significantly different ($P>0.05$) at high temperature, ambient temperature and low temperature. Hemoglobin at high temperature were 15.67 and 17.2 g/dl, respectively. Hemoglobin at ambient temperature were 14.32 and 19.27 g/dl, respectively. Hemoglobin at low temperature were 7.32 and 10.08 g/dl, respectively. Hemoglobin at low temperature showed significantly difference ($P<0.05$) with high and ambient temperature in both supplemental betaine and without betaine treatment.

The Na^+ observed from frog fed diets containing 0 and 1.5% betaine which incubated at different temperature for 6 hours showed no significantly difference ($P>0.05$) between these betaine levels. Na^+ of frog fed 0 and 1.5% betaine at high temperature were 131.33 and 132.25 mmol/l, respectively. Na^+ at ambient temperature were 131.50 and 133.00 mmol/l, Na^+ at low temperature were 115.50 and 109.50 mmol/l, Na^+ at low temperature showed significantly difference ($P<0.05$) with high and ambient temperature in both supplemental betaine and without betaine treatment.

The K^+ observed from frog fed diets containing 0 and 1.5% betaine which incubated at high temperature, ambient temperature and low temperature showed no significantly difference ($P>0.05$). The K^+ range from 4.04 - 5.55 mmol/l.

The Cl^- observed from frog fed diets containing 0 and 1.5% betaine which incubated at different temperature for 6 hours showed no significantly difference ($P>0.05$) between these betaine levels. Cl^- of frog fed 0 and 1.5% betaine at high temperature were 60.33 and 60.75 mmol/l, respectively. Cl^- at ambient temperature were 51.33 and 55.00 mmol/l, Cl^- at low temperature were 53.66 and 57.25 mmol/l, Cl^- at low temperature showed significantly difference ($P<0.05$) with high and ambient temperature in both supplemental betaine and without betaine treatment.

The pH observed from frog fed diets containing 0 and 1.5% betaine which incubated at high temperature, ambient temperature and low temperature showed no significantly difference ($P>0.05$). The pH range from 7.12 - 7.32.

Table 15 Hematological values and blood osmolarity of frog fed with pellet feed containing 0 and 1.5 % betaine challenge at different temperature

Parameter	Pellet feed							
	0 % betaine			1.5 % betaine			P.Value	Pool.SE
	40°C	27 °C	15 °C	40°C	27 °C	15 °C		
Osmolarity (mmo/kgH_20)	251.2 [a] ± 7.89	237.2 [a] ± 9.37	222.0 [b] ± 2.38	235.7 [a] ± 5.93	233.2 [a] ± 5.17	218.5 [b] ± 3.01	0.018	12.301
RBC ($x10^6/mm^3$)	0.17 [a] ± 0.01	0.18 [a] ± 0.05	0.26 [a] ± 0.02	0.19 [a] ± 0.01	0.19 [a] ± 0.04	0.20 [a] ± 0.05	0.593	0.079
HCT (%)	2.92 [a] ± 0.44	2.62 [a] ± 0.85	5.35 [a] ± 0.46	3.15 [a] ± 0.28	2.90 [a] ± 0.63	3.55 [a] ± 1.29	0.166	1.489
HGB (g/dl)	15.67 [a] ± 1.31	14.32 [a] ± 3.05	7.32 [b] ± 0.36	17.20 [a] ± 1.93	19.27 [a] ± 1.76	10.8[ab] ± 1.19	0.002	3.605
Na (mmol/l)	131.3[a] ± 3.38	131.5[a] ± 5.18	115.5[b] ± 2.72	132.2[a] ± 4.02	133.0[a] ± 3.71	109.5[b] ± 2.90	0.0007	7.445
K (mmol/l)	4.61[a] ± 1.38	4.01[a] ± 3.0	4.59 [a] ± 0.38	4.40 [a] ± 0.82	4.54 [a] ± 0.34	5.55 [a] ± 0.59	0.688	1.290
Cl (mmol/l)	60.33 [a] ± 1.45	51.33 [b] ± 0.88	53.66 [b] ± 1.20	60.75 [a] ± 0.85	55.00 [b] ± 1.15	57.2 [ab] ± 1.37	0.0003	2.163
pH	7.32 [a] ± 0.19	7.12 [a] ± 0.12	7.12 [a] ± 0.12	7.12 [a] ± 0.12	7.12 [a] ± 0.12	7.25 [a] ± 0.14	0.860	0.285

Note: [a,b] and [c] in the same row having the same superscript were not significantly different (P>0.05).

Water quality

During the experimental period, the temperatures were observed two times every day in the morning and after noon. The air temperature in the morning ranged from 26.00 – 31°C and in the after noon range from 28 – 32°C respectively. The minimum and maximum water temperature in the morning ranged from 24 – 28 °C and 25 – 29 °C respectively. The minimum and maximum water temperature in the after noon ranged from 25 – 29 °C and 27 – 30 °C respectively the graph of air temperature and water temperature during experimental period monitored showed in figure 9 and 10.

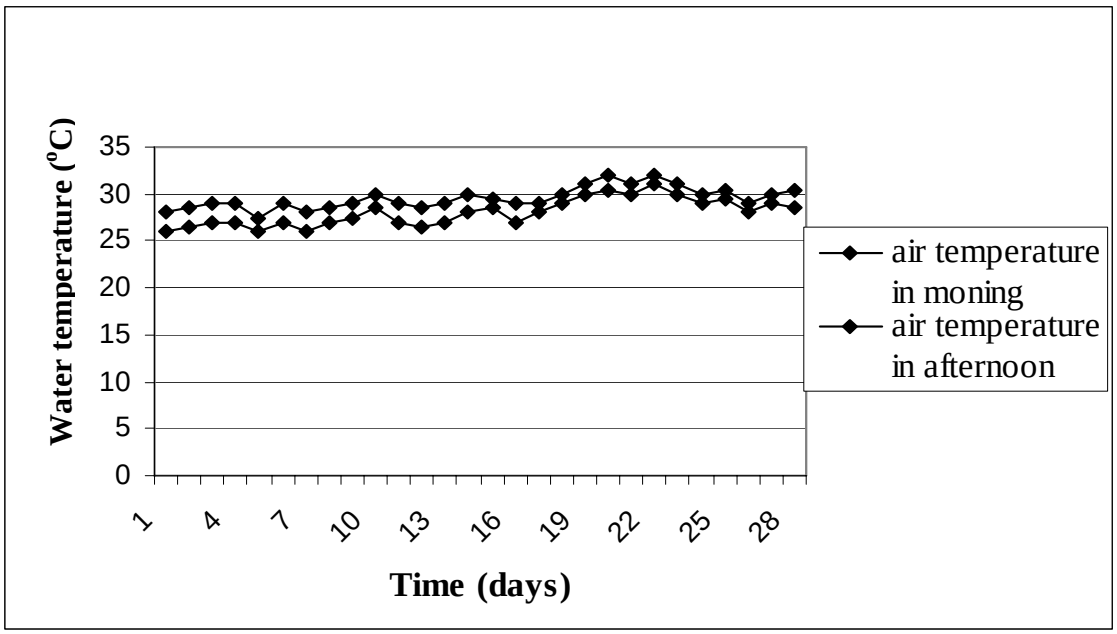
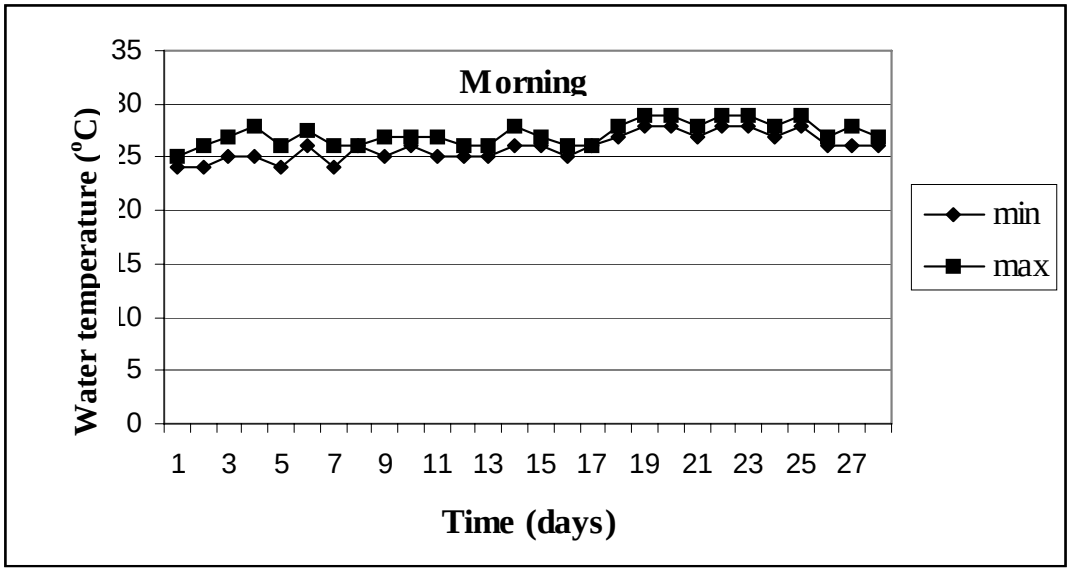


Figure 9 The indoor lab air temperature during culture period



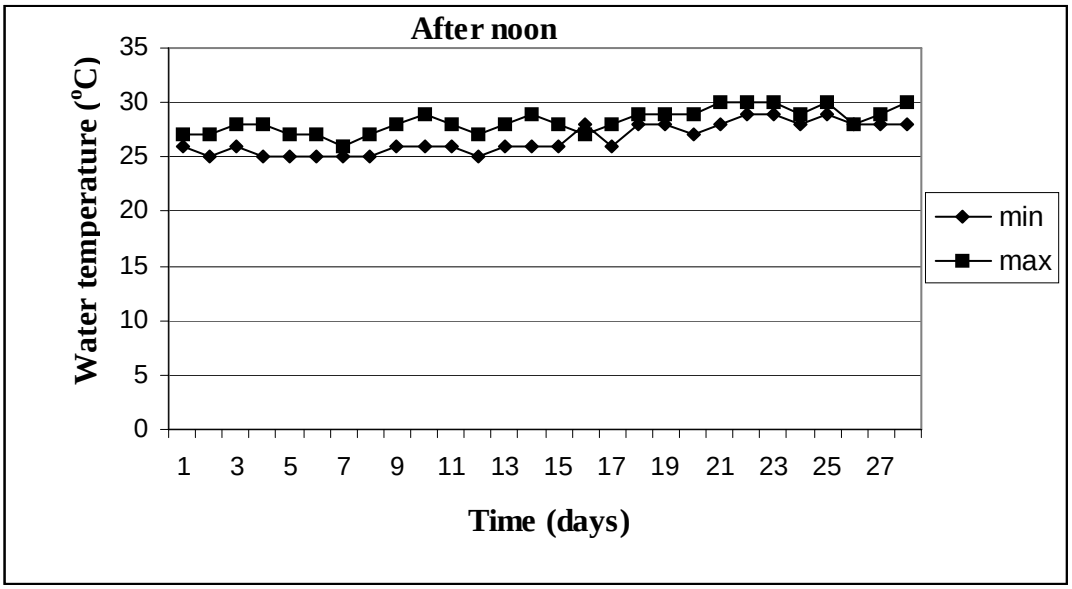


Figure 10 The minimum and maximum water temperature in frog tanks during culture period

During the experimental period, dissolved oxygen (DO), pH, orthophosphate total phosphate and BOD were monitored before and after change water. In culture tank before change water first time DO, pH, OrP, TP and BOD ranged from 0.75 – 0.9 mg/l, 6.5 – 7.1, 0.11 – 0.35 mg/l, 0.30 – 0.65 mg/l and 6.7 – 7.6 mg/l respectively . BOD after five days increased to 49 mg/l for moist feed 0% betaine and any treatments were error also can't measure. In culture tank after change water first time DO, pH, OrP, TP and BOD ranged from 1.35 – 1.39 mg/l, 6.8 – 7.0, 0.04 – 0.08 mg/l, 0.05 – 0.085 mg/l and 7.9 – 8 mg/l respectively. BOD after five days increased to 20 – 24 mg/l. In culture tank before change water second time DO, pH, OrP, TP and BOD ranged from 0.75 – 0.99 mg/l, 6.5 – 7.2, 0.10 – 0.39 mg/l, 0.38 – 0.82 mg/l and 8.0 – 8.4 mg/l respectively but BOD after five days increased to 44 -54 mg/l. In culture tank after change water second time DO, pH, OrP, TP and BOD ranged from 1.38 – 1.50 mg/l, 6.8 – 7.0, 0.03 – 0.05n mg/l, 0.04 – 0.07 mg/l and 8.0 – 8.5 mg/l respectively. BOD after five days increased to 23 -30 mg/l. The graphs of this parameter were shown in table 16.

The ammonia (NH_3-N) and nitrite (NO_2) concentration of water in the culture tank before changed water first time ranged from 0.06 – 0.1 mg/l and 0.42 – 0.59 mg/l respectively. In the culture tanks after changed water first time ranged from and 0.045 – 0.058 mg/l and 0.01 – 0.03 mg/l respectively. Ammonia and nitrite in the culture tanks before changed water second time during experiment ranged from 0.24 – 0.63 mg/l and 0.06 – 0.27 mg/l and respectively. In the culture tanks after changed water second time ranged from 0.01 – 0.05 mg/l and 0.02 – 0.03 mg/respectively.

Table 16 Water quality during period of frog culture (experiment 1)

Parameter	Experiment 1
Air temperature (^{o}C)	26 – 32
Water temperature (^{o}C)	24 – 30
DO (mg/l)	0.75 – 1.40
pH	6.5 – 7
NO_2 (mg/l)	0.01 – 0.27
NH_3 (mg/l)	0.04 – 0.63
Total P (mg/l)	0.05 – 0.82
Or P (mg/l)	0.03 – 0.35
BOD (mg/l)	6.7 - 51

Result of experiment II

The study on replacement fishmeal with golden apple snail meal in frog feed by varying percentage of snail meal substituted for protein from fishmeal 0, 50%, 100%, and 0% snail meal + 1.5% betaine were conducted. The research was divided into two trials. Firstly, research on young frog with size of 6 – 8 cm. and 20 – 30 g body weight and secondly, research on grower frog with size of 8 – 12 cm. and 60-75 g. The results of this research as follow:

Study on replacement fishmeal with golden apple snail meal in young frog

Growth performance

The growth performances of young frog fed different experimental treatment diets were evaluated. The results was shown in table 15 and Fig. 9. Mean initial weight of each treatment were not significantly differences ($P>0.05$). Mean final weight, mean weight gain, percentage of weight gain, average daily weight gain and specific growth rate of young frog showed significantly different ($P<0.05$) among treatments groups. Frog fed diet supplemental 50 and 100% snail meal for fish meal demonstrated the low growth performance than group of 0% snail meal both with and without betaine ($P<005$). Mean final weight were 58.19, 48.75, 64.44 and 64.86 g/frog, respectively. Mean weight gain were 32.75, 23.58, 39.11 and 40.25 g/frog, respectively. Percentage of weight gain was 128.64, 93.52, 154.20 and 163.73 %, respectively. Average daily weight gain were 1.17, 0.84, 1.39 and 1.43 g/frog/day, respectively and Specific growth rate were 2.95, 2.35, 3.32 and 3.45%/day, respectively in young frog fed diet supplemental 50 and 100% snail meal for fish meal and group of 0% snail meal both with and without betaine.

Table 17 Growth performance of young frog fed diet supplemental 50 and 100% snail meal for fish meal and group of 0% snail meal with and without betaine for 28 days (mean±SE)

Growth performance	Snail meal substituted for fishmeal in frog diet				P.value	Pool.SE
	50 %	100 %	0 %	0 %+1.5%bet		
Mean initial Weight(g)	25.44 [a] ± (0.45)	25.160 [a] ± (0.48)	25.33 [a] ± (0.25)	24.61 [a] ± (0.22)	0.445	0.645
Mean final Weight(g)	58.19 [ab] ± (2.10)	48.75 [b] ± (2.36)	64.44 [a] ± (3.96)	64.86 [a] ± (3.68)	0.021	5.431
Mean weight Gain (g)	32.75 [ab] ± (1.82)	23.58 [b] ± (1.93)	39.11 [a] ± (3.75)	40.25 [a] ± (3.80)	0.015	5.173
Percentage of weight gain (%)	128.64 [ab] ± (6.37)	93.52 [b] ± (6.32)	154.20 [a] ± (13.54)	163.73 [a] ± (16.30)	0.010	19.934
Average daily weight gain (g)	1.17 [ab] ± (0.06)	0.84 [b] ± (0.06)	1.39 [a] ± (0.13)	1.43 [a] ± (0.13)	0.014	0.182
Specific growth rate(%/day)	2.95 [a] ± (0.09)	2.35 [b] ± (0.11)	3.32 [a] ± (0.19)	3.45 [a] ± (0.22)	0.007	0.290
Survival rate (%)	97.25[a] (0.33)	94.42 [a] ± (0.33)	94.42 [a] ± (0.33)	100 [a] ± (0)	0.363	0.500

Note: [a,b] and [c] in the same row having the same superscript were not significantly difference (P>0.05)

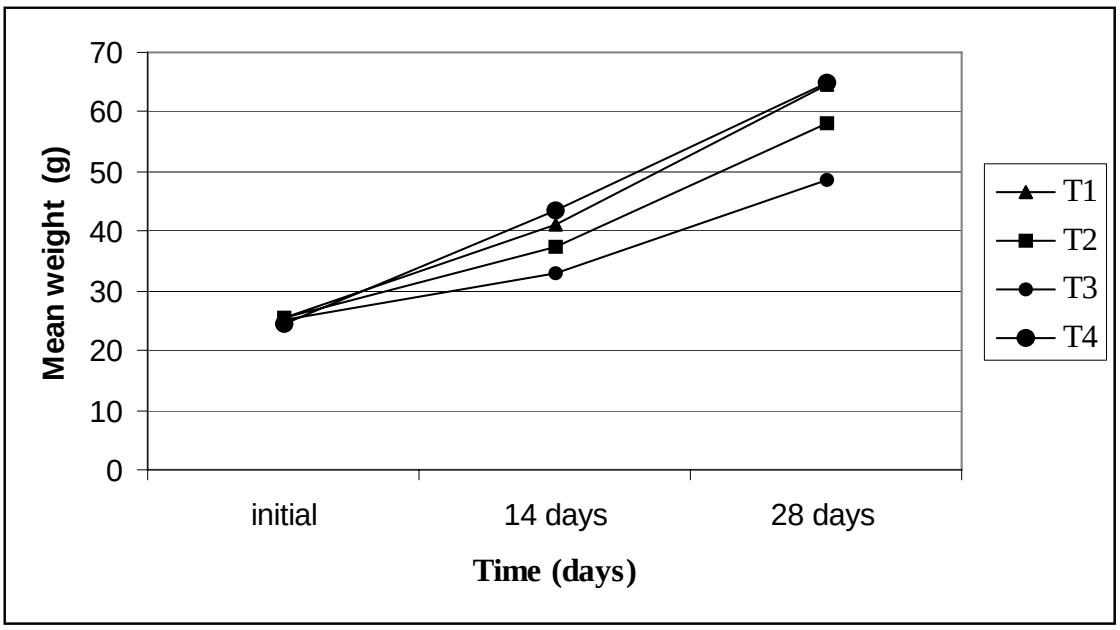


Figure 11 Growth performance of frog fed different diets

Note: T_1 = 0% snail meal with 0% betaine

T_2 = 50% snail meal

T_3 = 100% snail meal

T_4 = 0% snail meal with 1.5% betaine

Survival rate of young frog fed diet supplemental 50 and 100% snail meal for fish meal and group of 0% snail meal both with and without betaine showed no significantly difference ($p>0.05$). There were 97.25, 94.42, 94.42 and 100%, respectively. The survival rate of each period was presented in table 16 and figure 13.

Table 18 Survival rate of frog fed with different diets during 28 days

Time (days)	Snail meal substituted for fishmeal in frog diet			
	50 %	100 %	0 %	0 % + 1.5% bet
0	100.00	100.00	100.00	100.00
14	97.25	94.42	94.42	100.00
28	97.25	94.42	94.42	100.00

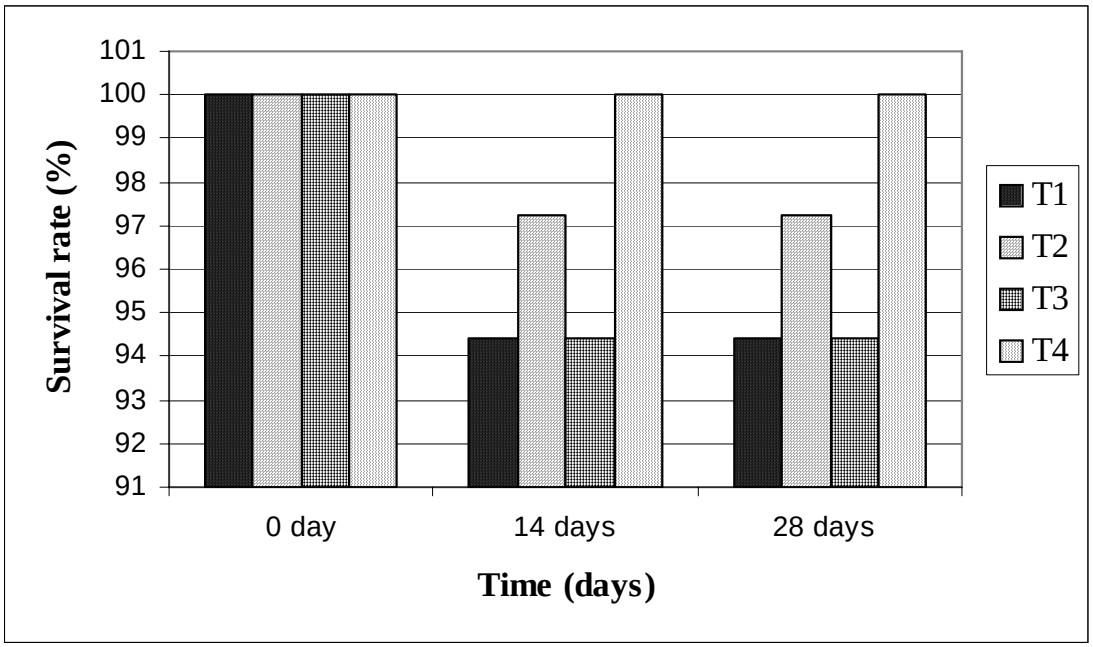


Figure 12 Survival rate of frog fed different diets during 28 days

Feed efficiency of the experimental frog

The feed efficiency of young frog fed diet supplemental 50 and 100% snail meal for fish meal and group of 0% snail meal both with and without betaine were investigated. The results showed in table 17. Feed intake of each treatment were not significantly differences ($P>0.05$). There was 1.71, 1.67, 1.75 and 1.71 g/frog/day, respectively. Percentage of feed intake, feed conversion ratio and protein efficiency ratio of young frog showed significantly different ($P<0.05$) among treatments groups. Frog fed diet supplemental 50 and 100% snail meal for fish meal demonstrated the low feed efficiency than group of 0% snail meal both with and without betaine. Percentage of feed intake was 1.02, 1.13, 0.97 and 0.95%, respectively. Feed conversion ratios were 1.47, 2.01, 1.27 and 1.20, respectively. Protein efficiency ratio were 0.74, 0.53, 0.89 and 0.91, respectively. in young frog fed diet supplemental 50 and 100% snail meal for fish meal and group of 0% snail meal both with and without betaine. Net protein utilization (NPU) of young frog in each treatment was in the same rang ($P>0.05$). There were 0.04, 0.03, 0.08 and 0.08, respectively.

Table 19 Feed efficiency of young frog fed different diets during 28 days (means± SE)

Feed efficiency	Snail meal substituted for fishmeal in frog diet				P.value	Pool.SE
	50 %	100 %	0 %	0 % +1.5%bet		
Feed intake (g/frog/day)	1.71 [a] ± (0.04)	1.67 [a] ± (0.05)	1.75 [a] ± (0.03)	1.71 [a] ± (0.05)	0.722	0.080
Percent feed intake (%)	1.02 [a] ± (0.04)	1.13 [a] ± (0.06)	0.97 [b] ± (0.02)	0.95 [b] ± (0.01)	0.059	0.071
Feed conversion ratio FCR	1.47 [a] ± (0.09)	2.01 [b] ± (0.20)	1.27 [a] ± (0.20)	1.20 [a] ± (0.08)	0.009	0.227
Protein efficiency ratio	0.74 [ab] ± (0.04)	0.53 [b] ± (0.04)	0.89 [a] ± (0.08)	0.91 [a] ± (0.08)	0.016	0.119
Net protein Utilization	0.04 [a] ± (0.01)	0.03 [a] ± (0.01)	0.08 [a] ± (0.04)	0.08 [a] ± (0.02)	0.469	0.048

Note: [a,b] and [c] in the same row having the same superscript are not significantly different (P>0.05)

Water quality

During the experimental period, the temperatures were observed two times every day in the morning and after noon. The air temperatures in the morning range from 23.00 – 28°C and in the after noon range 25 – 31 °C respectively. The minimum and maximum water temperature in the morning ranged from 22 – 27 °C and 23 – 28 °C respectively. The minimum and maximum water temperature in the after noon ranged from 24 – 28 °C and 24 – 30 °C respectively the graph of air temperature and water temperature during experimental period monitored showed in figure 13 and 14.

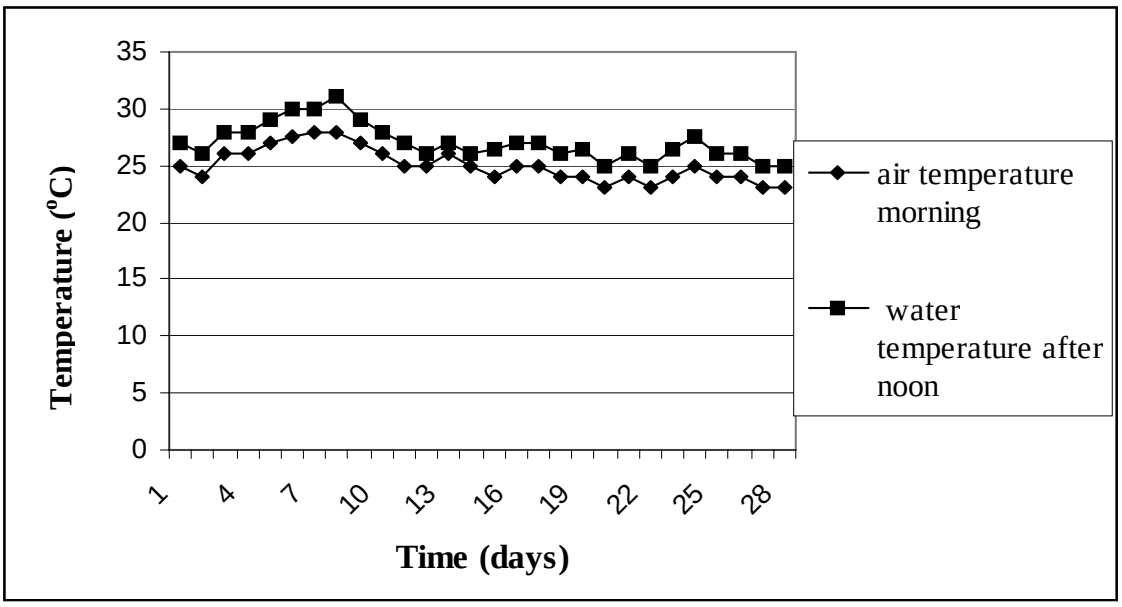
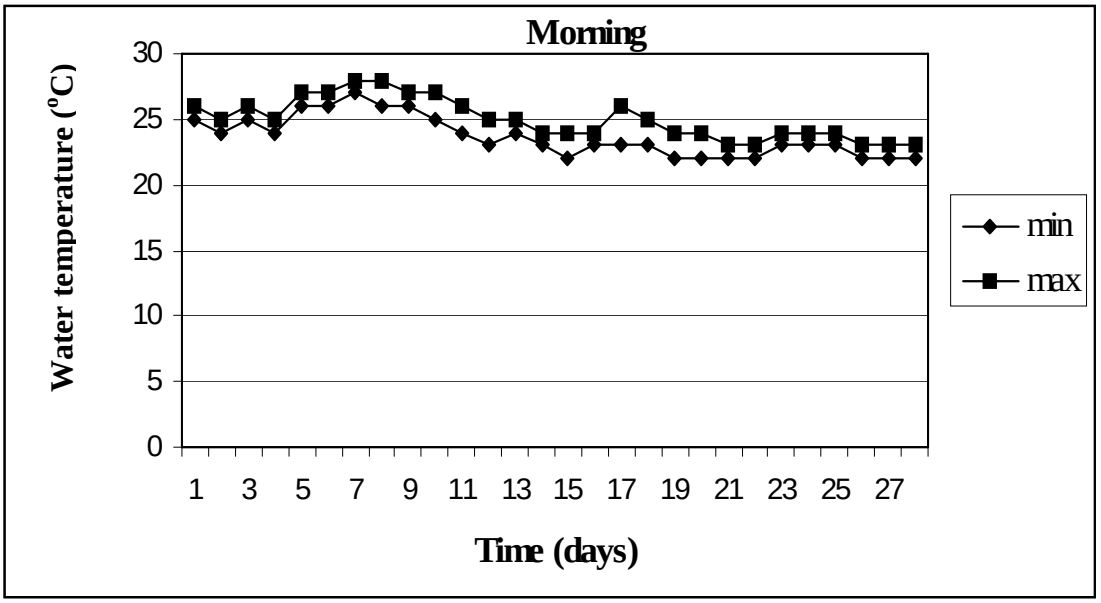


Figure 13 The air temperature indoor lab during culture period (experiment 2.1)



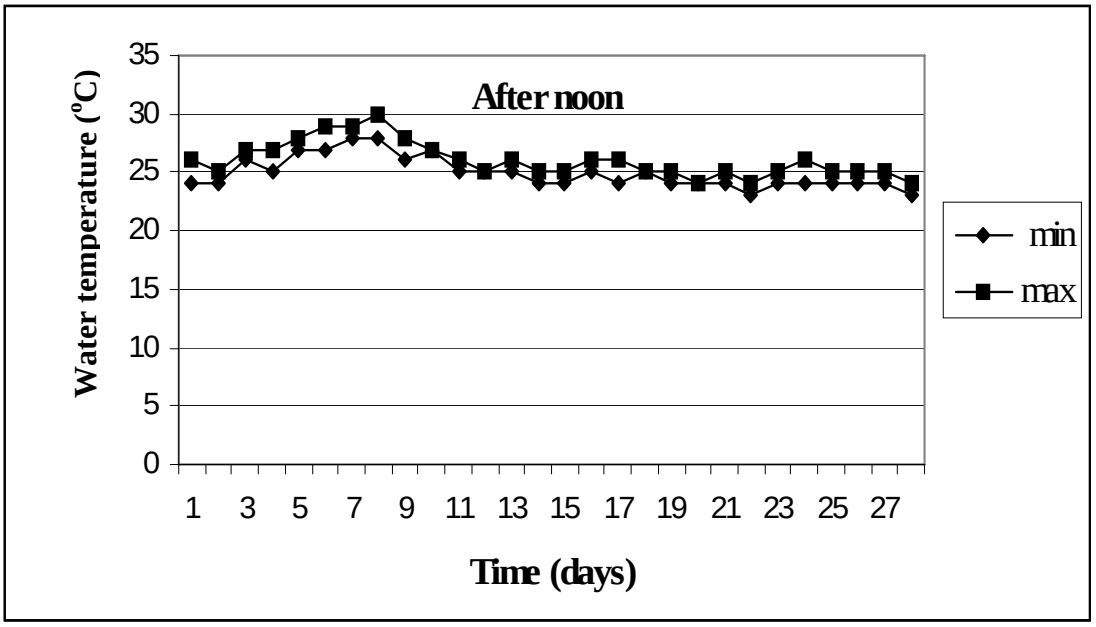


Figure 14 The minimum and maximum water temperature in the tanks during culture period (experiment 2.1)

During the experimental period, dissolved oxygen (DO), pH, orthophosphate, total phosphate and BOD were monitored before and after change water. In culture tank before change water first time DO, pH, OrP, TP and BOD ranged from 0.53 – 0.70 mg/l, 6.5 – 7.0, 0.12 – 0.35 mg/l, 0.32 – 0.43mg/l and 7.7 – 8.0 mg/l respectively. BOD after five days increased to 32 – 42 mg/l. In culture tank after change water first time DO, pH, OrP, TP and BOD ranged from 1.45 – 1.58 mg/l, 7.0 – 7.2, 0.04 – 0.07n mg/l, 0.056 – 0.079 mg/l and 8.0 – 8.3 mg/l respectively. BOD after five days increased to 21 - 25 mg/l. In culture tank before changed water second time DO, pH, OrP, TP and BOD ranged from 0.48 – 0.68 mg/l, 6.5 – 7.2, 0.12 – 0.32 mg/l, 0.37 – 0.43 mg/l and 7.8 – 8.2 mg/l respectively. BOD after five days increased to 34 - 42 mg/l. In culture tank after changed water second time DO, pH, OrP, TP and BOD ranged from 1.52 – 1.70 mg/l, 6.7 – 7.3, 0.035 – 0.058n mg/l, 0.054 – 0.064 mg/l and 8.0 – 8.8 mg/l respectively. BOD after five days increased to 29 – 32 mg/l. The graphs of this parameter were shown in table 20.

The ammonia (NH_3-N) and nitrite (NO_2) concentration of water in the culture tank before changed water first time ranged from 0.46 – 0.55 mg/l and 0.069 – 0.120 mg/l respectively. In the culture tanks after changed water first time ranged from and 0.018 – 0.032 mg/l and 0.041 – 0.056 mg/l respectively. Ammonia and nitrite in the culture tanks before changed water second time during experiment ranged from 0.46 – 0.61 mg/l and 0.070 – 0.107 mg/l and respectively. In the culture tanks after changed water second time ranged from 0.035 – 0.046 mg/l and 0.034 – 0.046 mg/l respectivel

Table 20 Water quality during frog culture period (experiment 2.1)

Parameter	Experiment 2.1
Air temperature (^{o}C)	23 – 31
Water temperature (^{o}C)	22 – 30
DO (mg/l)	0.48 – 1.70
pH	6.5 – 7.2
NO_2 (mg/l)	0.01 – 0.12
NH_3 (mg/l)	0.04 – 0.61
Total P (mg/l)	0.05 – 0.43
Or P (mg/l)	0.03 – 0.37
BOD (mg/l)	7.5 - 42

Study on replacement fishmeal with golden apple snail meal in grower frog

Growth performance

The growth performances of grower frog fed different experimental treatment diets were evaluated. The results showed in table 19 and fig. 17. Mean initial weight of each treatment were not significantly differences ($P>0.05$). Mean final weight, mean weight gain, percentage of weight gain, average daily weight gain and specific growth rate of grower frog showed significantly different ($P<0.05$) among treatments groups. Frog fed diet supplemental 50 and 100% snail meal for fish meal demonstrated the same ($P>0.05$) growth performance as group of 0% snail meal both with and without betaine. Mean final weight were 177.07, 155.61, 172.16 and 181.20g/frog, respectively. Mean weight gain were 107.78, 87.06, 102.25 and 111.64

g/frog, respectively. Percentage of weight gain was 155.45, 126.93, 154.20 and 160.41 %, respectively. Average daily weight gain were 2.56, 2.07, 2.44 and 2.66 g/frog/day, respectively and Specific growth rate were 2.23, 1.95, 2.14 and 2.28 %/day, respectively in grower frog fed diet replacement of 50 and 100% snail meal for fish meal and group of 0% snail meal both with and without betaine.

Table 21 Growth performance of grower frog fed diet supplemental 50 and 100% snail meal for fish meal and group of 0% snail meal with and without betaine for 42 days (means ± SE)

Growth performance	Snail meal substituted for fishmeal in frog diet				P.value	Pool.SE
	50 %	100 %	0 %	0% +1.5%bet		
Mean initial Weight(g)	69.29 [a] ± (0.80)	68.55 [a] ± (0.77)	69.66 [a] ± (0.83)	69.55 [a] ± (0.48)	0.719	0.753
Mean final Weight(g)	177.07 [a] ± (5.27)	155.61 [a] ± (3.93)	172.16 [a] ± (10.25)	181.20 [a] ± (6.16)	0.116	11.826
Mean weight Gain (g)	107.78 [a] ± (4.49)	87.06 [a] ± (3.19)	102.25 [a] ± (10.04)	111.64 [a] ± (5.68)	0.105	11.078
Percent of weight gain (%)	155.45 [a] ± (4.79)	126.93 [a] ± (3.34)	154.20 [a] ± (13.54)	160.41 [a] ± (7.10)	0.088	14.502
Average Daily weight gain (g)	2.56 [a] ± (0.10)	2.07 [a] ± (0.07)	2.44 [a] ± (0.24)	2.66 [a] ± (0.13)	0.105	0.264
Specific growth rate(%/day)	2.23 [a] ± (0.04)	1.95 [a] ± (0.03)	2.14 [a] ± (0.13)	2.28 [a] ± (0.06)	0.085	0.062
Survival rate (%)	93.33 [a] (3.85)	95.55 [a] ± (2.22)	93.33 [a] ± (3.85)	97.77 [a] ± (2.22)	0.718	0.965

Note: [a,b] and [c] in the same row having the same superscript are not significantly different (P>0.05)

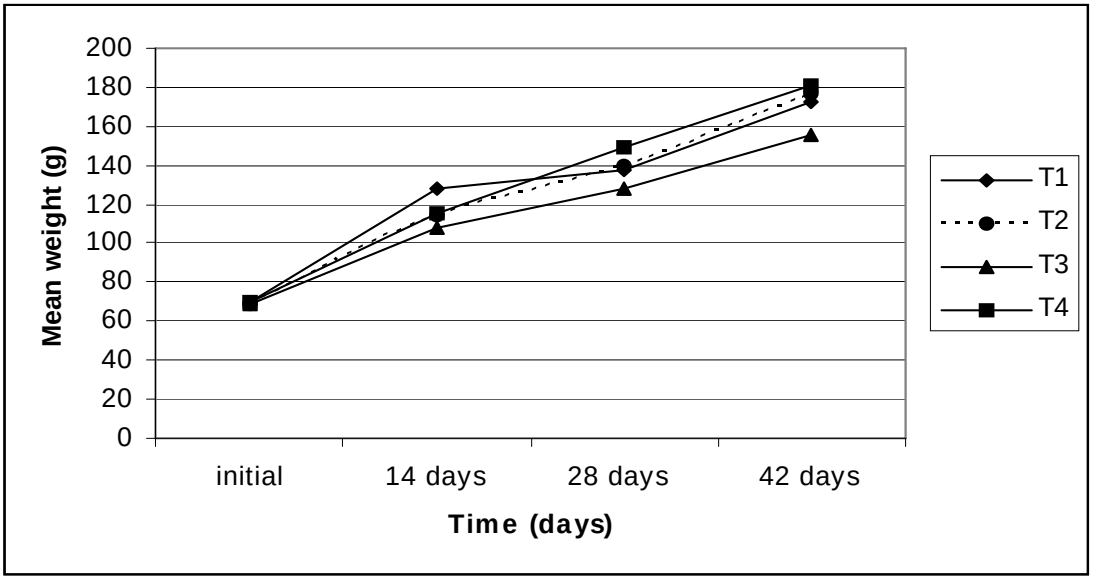


Figure 15 Growth performance of grower frog fed different diets

Note: T_1 = 0 % snail meal with 0% betaine

T_2 = 50% snail meal

T_3 = 100% snail meal

T_4 =0 % snail meal with 1.5% betaine

Survival rate of grower frog fed diet supplemental 50 and 100% snail meal for fish meal and group of 0% snail meal both with and without betaine showed no significantly difference ($P>0.05$). There were 93.33, 95.55, 95.55 and 97.77%, respectively. The survival rate of each period was presented in table 20 and figure 16.

Table 22 Survival rate of frog fed with different diets during 42 days

Time (days)	Snail meal substituted for fishmeal in frog diet			
	50 %	100 %	0 %	0 % + 1.5% bet
0	100.00	100.00	100.00	100.00
14	100.00	100.00	100.00	100.00
28	93.33	95.55	95.55	97.77
42	93.33	95.55	95.55	97.77

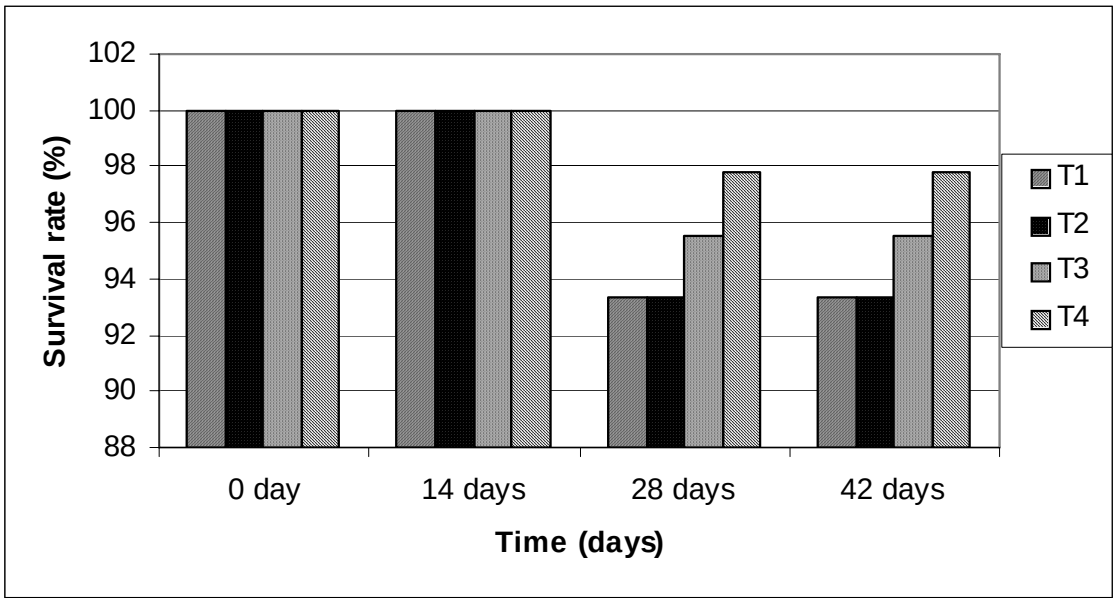


Figure 16 Survival rate of grower frog fed different diets during 42 days

Note: T_1 = 0 % snail meal with 0% betaine

T_2 = 50% snail meal

T_3 = 100% snail meal

T_4 = 0 % snail meal with 1.5% betaine

Feed efficiency of the experimental frog

The feed efficiency of grower frog fed diet supplemental 50 and 100% snail meal for fish meal and group of 0% snail meal both with and without betaine were investigated. The result was shown in table 21. Feed intake of each treatment were not significantly differences (P>0.05). There was 4.36, 4.37, 4.92 and 3.86 g/frog/day, respectively. Percentage of feed intake, feed conversion ratio and net utilization protein of each treatment also was not significantly differences (P>0.05). There were 0.88, 0.97, 1.02 and 0.77%; 1.70, 2.11, 2.06 and 1.47; 0.04, 0.12, 0.18 and 0.06 respectively. The protein efficiency ratio of grower frog showed not significantly different (P>0.05) among treatments groups. There were 2.45, 1.99, 2.33 and 2.54%, respectively.

Table 23 Feed efficiency of grower frog fed different diets during 42 days (means± SE)

Feed efficiency	Snail meal substituted for fishmeal in frog diet				P.value	Pool.SE
	50 %	100 %	0 %	0 % +1.5%bet		
Feed intake (g/frog/day)	4.36 [a] ± (0.66)	4.37 [a] ± (0.10)	4.92 [a] ± (0.08)	3.86[a] ± (0.88)	0.628	0.965
Percent feed intake (%)	0.88 [a] ± (0.13)	0.97 [a] ± (0.02)	1.02 [a] ± (0.06)	0.77 [a] ± (0.18)	0.506	0.035
Feed conversion ratio FCR	1.7 0 [a] ± (0.26)	2.11 [a] ±(0.08)	2.06 [a] ± (0.23)	1.47 [a] ± (0.35)	0.303	0.439
Protein efficiency ratio (%)	2.45 [a] ± (0.10)	1.99 [a] ± (0.07)	2.33 [a] ± (0.22)	2.54 [a] ± (0.12)	0.110	0.252
Net protein Utilization (%)	0.04 [a] ± (0.01)	0.12 [a] ± (0.03)	0.18 [a] ± (0.06)	0.06 [a] ± (0.03)	0.167	0.070

Note: [a,b] and [c] in the same row having the same superscript are not significantly different (P>0.05)

Hematology and blood osmolarity

The hematological value and blood osmolarity of experimental frog fed different betaine level and snail meal feed were collected at the end of study. The result was presented in tables 22 and 23. The parameter such as osmolarity, red blood cell, hematocrit, and hemoglobin, Na, K and Cl were determined.

The osmolarity of frog fed diets containing 0% SM and 0% SM+1.5%B at high temperature, ambient temperature and low temperature were 229, 237, 229 and 244, 233, 244 mmol/kgH_2O, respectively. The osmolarity of frog fed diets containing 50% SM and 100% SM at high temperature, ambient temperature and low temperature were 231, 227, 239 and 231, 218,210 mmol/kgH_2O, respectively.

The red blood cells (RBC) of frog fed diets containing 0% SM and 0% SM +1.5%B at high temperature, ambient temperature and low temperature were 0.09, 0.08, 0.07 and 0.11, 0.09, 0.08 x10^6 cell/ml, respectively. The RBC of frog fed diets containing 50% SM and 100% SM at high temperature, ambient temperature and low temperature were0.12, 0.07, 0.08 and 0.14, 0.09, 0.10 x10^6 cell/ml, respectively.

The hemoglobin of frog fed diets containing 0% SM and 0% SM +1.5%B at high temperature, ambient temperature and low temperature were 7.85, 6.20, 8.00 and 11.05, 9.2, 8.95 g/dl, respectively. The hemoglobin of frog fed diets containing 50% SM and 100% SM at high temperature, ambient temperature and low temperature were 10.05, 6.2, 7.15 and 6.95, 9.20 and 8.45 g/dl, respectively.

The hemotocrite of frog fed diets containing 0% SM and 0%SM +1.5%B at high temperature, ambient temperature and low temperature were 1.50, 1.50, 1.50 and 2.00, 2.00, 1.50%, respectively. The hemotocrite of frog fed diets containing 50% SM and 100% SM at high temperature, ambient temperature and low temperature were 2.50, 1.00, 1.50 and 3.00, 2.00, 2.00%, respectively.

The Na^+ of frog fed diets containing 0% SM and 0%SM +1.5%B at high temperature, ambient temperature and low temperature were 101, 115,116 and 111, 120,111 mmol/l, respectively. The Na^+ of frog fed diets containing 50% SM and 100% SM at high temperature, ambient temperature and low temperature were 110, 104,101 and 107, 108, 106 mmol/l, respectively.

The K^+ of frog fed diets containing 0% SM and 0%SM +1.5%B at high temperature, ambient temperature and low temperature were 4.6, 4.0, 3.8 and 3.9, 2.5, 4.1 mmol/l, respectively. The K^+ of frog fed diets containing 50% SM and 100% SM at high temperature, ambient temperature and low temperature were 4.9, 3.5, 3.3 and 6.3, 3.5, 5.1 mmol/l, respectively.

The Cl^- of frog fed diets containing 0% SM and 0%SM +1.5%B at high temperature, ambient temperature and low temperature were 65, 72, 65 and 74, 77,74 mmol/l, respectively. The Cl^- of frog fed diets containing 50% SM and 100% SM at high temperature, ambient temperature and low temperature were 75, 72, 75 and 67, 75,67 mmol/l, respectively.

Table 24 Blood osmolality of frog fed 0% and 1.5 % betaine feed challenge at different temperature

parameter	Pellet feed					
	0 % Snail meal			0 % Snail meal + 1.5%betaine		
	40 °C	27 °C	15 °C	40 °C	27 °C	15 °C
Osmolarity (mmo/kgH_20)	229	237	229	244	233	244
RBC (x 10^6/mm^3)	0.09	0.08	0.07	0.11	0.09	0.08
HCT (%)	1.50	1.50	1.50	2.00	2.00	1.50
HGB (g/dl)	7.85	6.20	8.00	11.05	9.20	8.95
Na (mmol/l)	101	115	116	111	120	111
K (mmol/l)	4.60	4.00	3.80	3.90	2.50	4.10
Cl (mmol/l)	65.00	72.00	65.00	74.00	77.00	74.00

Table 25 Blood osmolality of frog fed 50% and 100 % snail meal in feed challenge at different temperature

parameter	Pellet feed					
	0 % Snail meal			0 % Snail meal + 1.5%betaine		
	40 °C	27 °C	15 °C	40 °C	27 °C	15 °C
Osmolarity (mmo/kgH_20)	231	227	229	231	218	210
RBC (x 10^6/mm^3)	0.12	0.07	0.08	0.14	0.09	0.10
HCT (%)	2.50	1.00	1.50	3.00	2.00	2.00
HGB (g/dl)	10.05	6.20	7.15	6.95	9.20	8.45
Na (mmol/l)	110	104	101	107	108	106
K (mmol/l)	4.90	3.50	3.30	6.30	3.50	5.10
Cl (mmol/l)	75.00	72.00	75.00	67.00	75.00	67.00

Water quality

During the experimental period, the temperatures were observed two times every day in the morning and after noon. The air temperature in the morning range from 23.00 – 28°C and in the after noon range from 25 – 31 °C respectively. The minimum and maximum water temperature in the morning ranged from 22 – 27 °C and 23 – 28 °C respectively. The minimum and maximum water temperature in the after noon ranged from 24 – 28 °C and 24 – 30 °C respectively. The graph of air temperature and water temperature during experimental period monitored showed in fig 17 and 18.

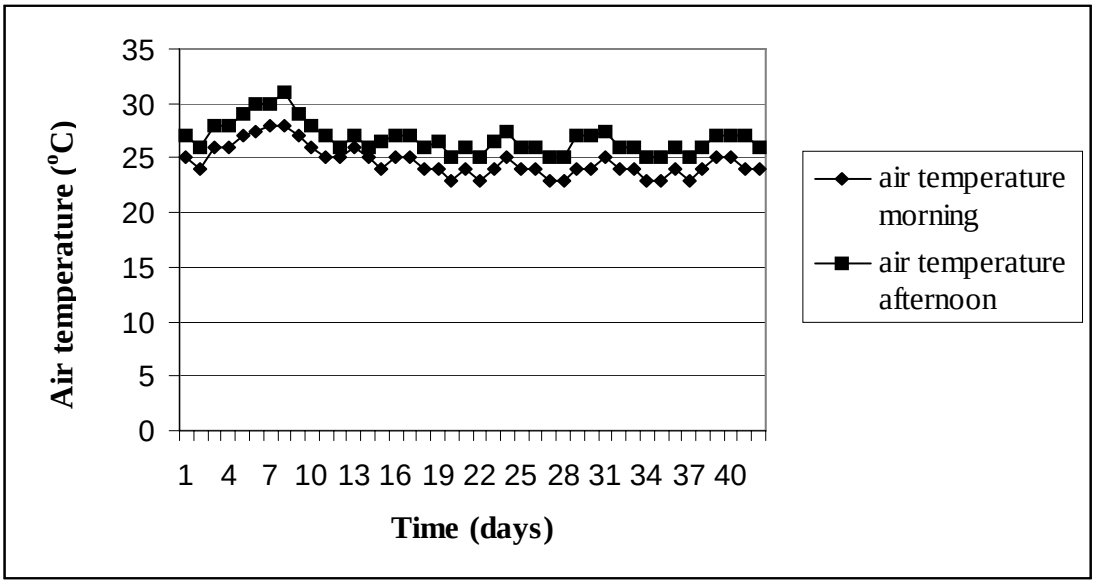
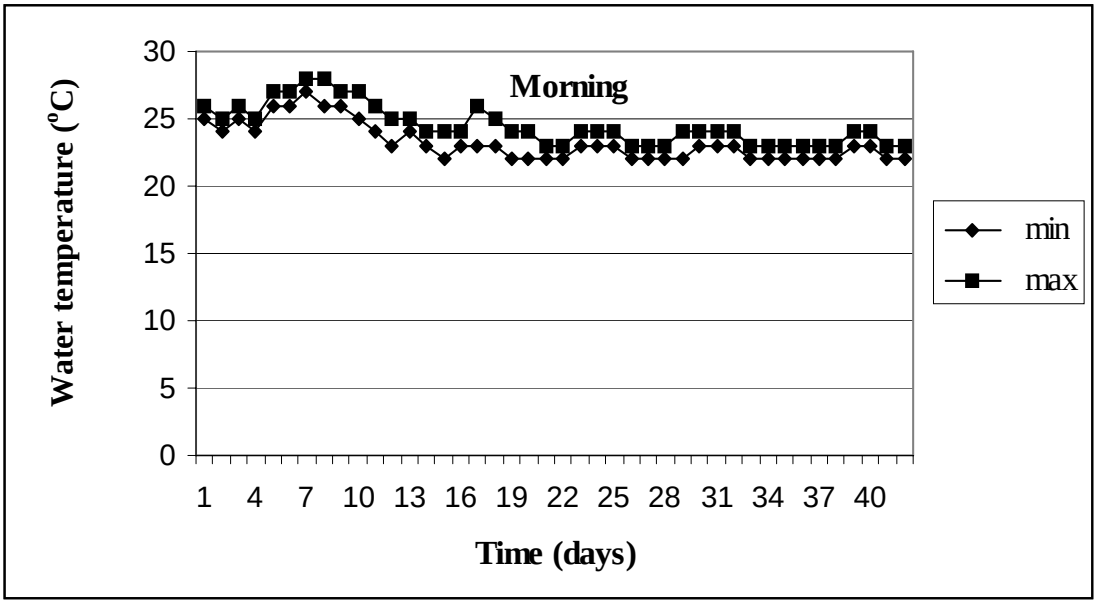


Figure 17 The indoor lab air temperature during culture period (experiment 2.2)



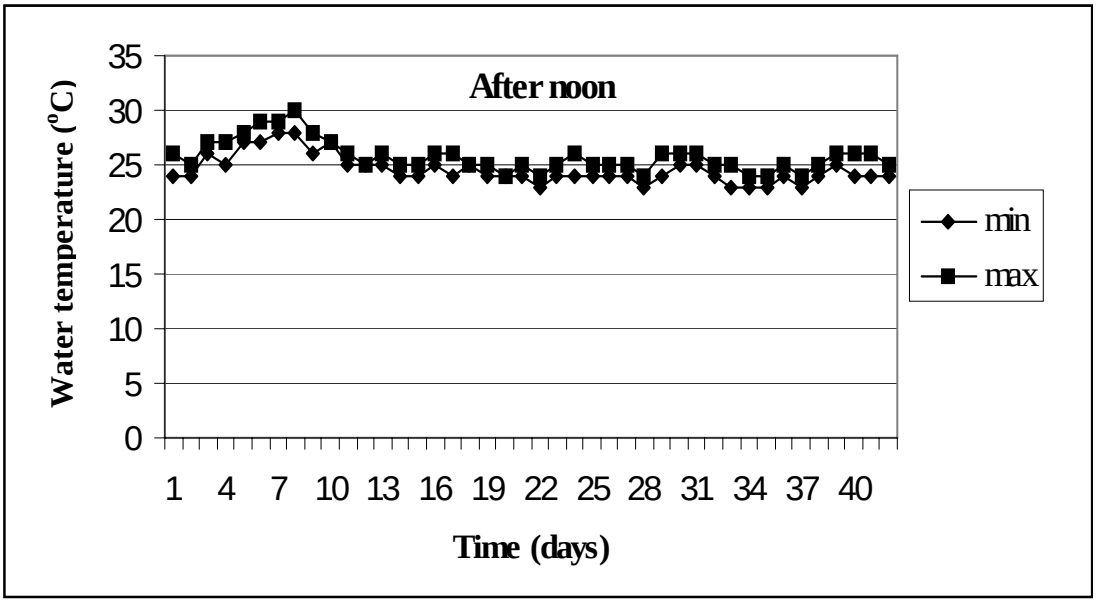


Figure 18 The minimum and maximum water temperature during culture period (experiment 2.2)

During the experimental period, dissolved oxygen (DO), pH, orthophosphate, total phosphate and BOD were monitored before and after change water. In culture tank before change water first time DO, pH, OrP, TP and BOD ranged from 0.40 – 0.60 mg/l, 6.8 – 7.2, 0.17 – 0.28 mg/l, 0.37 – 0.50 mg/l and 6.7 – 7.2 mg/l respectively but BOD after five days increased ranged 40 – 46 mg/l. In culture tank after change water first time DO, pH, OrP, TP and BOD ranged from 1.50 – 1.6 mg/l, 6.8 – 7.2, 0.04 – 0.06 mg/l, 0.056 – 0.063 mg/l and 7.4 – 8.3 mg/l respectively but BOD after five days increased ranged 16 - 27 mg/l.

In culture tank before changed water second time DO, pH, OrP, TP and BOD ranged from 0.32 – 0.42 mg/l, 6.8 – 7.2, 0.18 – 0.32 mg/l, 0.37 – 0.51 mg/l and 7.2 – 8.0 mg/l respectively but BOD after five days increased ranged 33 – 40 mg/l. In culture tank after changed water second time DO, pH, OrP, TP and BOD ranged from 1.56 – 1.75 mg/l, 6.8 – 7.1, 0.037 – 0.051 mg/l, 0.051 – 0.06 mg/l and 7.7 – 8.2 mg/l respectively but BOD after five days increased ranged 22 – 35 mg/l. The graphs of this parameter were shown in table 25.

The ammonia (NH_3-N) and nitrite (NO_2) concentration of water in the culture tank before changed water first time ranged from 0.57 – 0.99 mg/l and 0.099 – 0.13 mg/l respectively. In the culture tanks after changed water first time ranged from and 0.043 – 0.057 mg/l and 0.025 – 0.034 mg/l respectively. Ammonia and nitrite in the culture tanks before changed water second time during experiment ranged from 0.58 – 0.93 mg/l and 0.070 – 0.12 mg/l and respectively. In the culture tanks after changed water second time ranged from 0.039 – 0.044 mg/l and 0.036 – 0.042 mg/l respectively.

Table 26 Water quality during period of frog culture (experiment 2.2)

Parameter	Experiment 2.2
Air temperature (^{o}C)	23 – 31
Water temperature (^{o}C)	22 – 30
DO (mg/l)	0.32 – 1.75
pH	6.8 – 7.2
NO_2 (mg/l)	0.02 – 0.13
NH_3 (mg/l)	0.04 – 0.99
Total P (mg/l)	0.05 – 0.51
Or P (mg/l)	0.03 – 0.32
BOD (mg/l)	7.0 - 46

DISCUSSIONS

The studies on using golden apple snail meal and betaine in frog feed were conducted. The objectives of the present study were to determined suitable form of frog feed, optimum level of golden apple snail meal for replacing fishmeal and optimum betaine level in diets that would enable lowland frog to promote growth performance. The study on the attractant effect of betaine and effect of betaine on temperature stress tolerance also investigate. The result demonstrated that pelleted form showed the better growth performance than moist feed ($P<0.05$), high feed intake and better protein efficiency ratio because pellet was dry form and high water stability. Feed lost during consumption was low; hence, feed efficiency was better. Although, moist feed was soft, high palatibility and easy to consume but it cause high feed lost then effect on water quality and resulting on adverse feed efficiency. This agreed with Chirdchan *et al* (1995) which reported that frog feed should be pellet feed than moist feed. Moist feed when feeding long time would form a strong diet and when it wet, it loss then frog can not eat. On the other hands, generally, frog prefer living prey than non living prey such as pellet feed and moist feed. When frog consume pellet feed, pellet can move in its mouth but moist feed can not (Khamsivilay and Phanousith , 2002).

The effect of supplemental betaine in frog diets show low responsibility on growth performance that agree with effect on marine shrimp (Ketsada, 2005; Chiariya, 2005; Adith, 2005). These results did not agree with Felix and Duhasan (2004) in *(Macrobrachium rosenbergii)* and not agree with Hildan *et a*l (1998) in salmon. The supplemental glycien and betaine 1-3% in prawn and salmon diet presented the high weight gain, high specific growth rate, high feed intake and good FCR compared control.

In young frog, effect of betaine showed low responsibility on temperature stress tolerance, this might be frog was pokilotherm animal, they can control their osmolarity and ions level in condition of high and ambient temperature better than in cool condition.

Young frog able to use golden apple snail meal replacing for fishmeal less than 50% in frog diets because of growth performance and feed efficiency reduced. These related to the amino acid levels in fishmeal was higher than snail meal especially methionine. Fish meal contained methionine 2.4 – 3.0% where as snail meal contained methionine 0.89% (Hertrampf and Piedad-Pascual, 2000). Then, feed conversion and feed efficiency showed low feed quality which related to protein efficiency ratio. (Millamena, 1994; Bombeo-Tuburan, 1995; Boonyaratpalin and William, 2002; Webster and Lim, 2002). Although requirement of essential amino acid in frog was not clearly but frog require methionine look like other carnivorous animal. Protein digestibility in young frog was not as good as grower frog, hence, crude protein (40%) in feed composed from fish meal was enough for amino acid requirement but substitution of snail meal 50% of protein from fishmeal could not provide enough amino acid for young frog.

Supplemental betaine 1.5% in young frog feed showed no effect on growth performance and also on feed efficiency. High protein level in feed might be provided amino acid enough for being attractant in feed.

Grower frog able to use golden apple snail meal replacing for fishmeal up to 100% in frog diets without any adverse effects. This related to the digestibility in grower frog (animals) was higher than young frog (animals). The level of crude protein (40%) in feed was enough for fulfill amino acid requirement when substitution snail meal for fishmeal in frog diet. Pichsmay (2001) reported that protein levels for lowland frog range between 30 to 45%.

Supplemental betaine 1.5% in grower frog showed no effect on growth performance and also showed no effect on feed efficiency that cause by the high protein level provided amino acid enough for being attractant in feed but for osmo-protectant effects, supplemental betaine 1.5% for 2 month tended to increase osmolarity, chloride ion in frog blood and also increase hematological values (red blood cell, hemoglobin and hematocrit). Therefore frogs can tolerance during temperature change both in high and cool temperature.

CONCLUSIONS

The study on using golden apple snail meal and betaine in frog feed were investigate for determined form of frog feed, optimum level of golden apple snail meal for replacing fishmeal and optimum betaine level in diets. The study on the attractant effect of betaine and effect of betaine on temperature stress tolerance also investigate. The research demonstrated as following:

1. Pelleted feed showed the better growth performance than moist feed ($p<0.05$)

2. The effect of supplemental betaine in frog diets showed low responsibility on growth performance and feed efficiency.

3. In young frog, effect of betaine on temperature stress tolerance showed low responsibility in high (40°C), ambient (27°C) and cool condition (15°C).

4. Young frog able to use golden apple snail meal replacement for fishmeal less than 50% in frog diets but grower frog could use up to 100% in frog diets without any adverse effects.

5. Supplemental betaine 1.5% in grower frog showed no effect on growth performance and feed efficiency

6. Osmo-protectant effects of supplemental betaine 1.5% for 2 month tended to increase osmolarity, chride ion in frog blood and also increase hematological value (red blood cell, hemoglobin and hematocrit) then frog can tolerate temperature change both in high and cool temperature.

LITERATURE CITED

AOAC. 2000. **Official Method of Analysis of the Association of the Official Analysis Chemists.** 17th edition. Association of the Official Analysis Chemists, Inc,. Arlington, Virginia. 1298 pp.

Amatyakul, C. B. Chaopaknum. C. Udomkarn. S. Sumanochitrapron. P. Sripatprasite. U. Iimsilp and D. Nantamongkolgul. 1995. **Common lowland frog *Rana rugulosa***, Inland Fisheries Division, Department of Fisheries, Ministry of Agriculture and Cooperatives .pp. 1-21. (in Thai)

Alternative Medicine Reviews. 2003. **Monograph Betaine**, Thorne Research, Inc. 1993-1995 pp.

APHA. 1989. **Standard Method for Examination of Water and Wastewater**. 20th edition. American Public Health Association, American Water work Association and Water Pollution Control Federation, Washington DC, USA

Adith. S. 2005. Effect of Betaine on Stress Tolerance from Salinity Change and Acute Ammonia Toxicity in Black Tiger shrimp (*Penaeus monodon*, Fabricius) Post Larvae (P_{15-60}). **The Proceedings of 44th Kasetsart University Annual Conference**. pp. 612 – 619.

Bombeo – Tuburan, I., S. Fukumoto and E. M. Rodriguez. 1995. Use of golden apple snail, cassava and maize as feeds for tiger shrimp, *Penaeus monodon*, in pond. **Aquaculture** 131: 90 – 100.

Boonyaratpalin, M and K. William. 2002. Asian sea bass *Latescal carifer*. *In* Webster, C. D and C. E. Lim (eds) **Nutrient Requirement and Feeding of Finfish for Aquaculture.** CABI. Publishing. CAB International Wallingford Oxon. UK. pp. 40 - 50.

Boonpran, M. V. Thansalanoukith and P, Suthaninat. 1993. **Frog culture Technology Cooperative Thai – Japan** P, Bangkok. 35 pp.

Boonmanch, S. S, Keomany and V, Keoloued. 1999. Study on Protein Requirement in Diet for Bullfrog, Faculty Agriculture, Ubon Ratchathani University. pp. 2-10. (in Thai).

Briefing**.** 1996. **Cultor Ltd**. **Finnsugar Bioproducts,** Helsinki, FINLAND p 11-18.

Canphen, E. 1987. **Frog culture Knowledge and Technical** in Frog Breeding and Frog culture, Company People Ltd. Bangkok, Thailand. pp. 50-54.

Caguan, A.G and L. S. Doria. 1989. Golden Apple Snail *Pomacea insularis canalicuta* meal as fed for Nile tilapia *Oreochromis niloticus* fingering in aquarium. **CLSU Scientific Journal.** 9(3): 24 – 29.

Caguan, A.G and R. C. Joshi. 2002. Golden Apple Snail *Pomacea spin* the Philippines. **Paper Presented at the ICMAM Ppecial Working Group on Golden Apple Snail.** 10 p.

Chithtaprapong, T and P. Seetasith. 1985. **Using Feed Formula Different Journal Fisheries** 41 (6): 589 – 592.

Chiariya, S. 2005. Effect of Betaine on Growth performances Digestive Enzymes Activity and Protein Synthesis in Giant Tiger Shrimp (*Penaeus monodon*, Fabricius) Post Larvae (P_{15-60}). **The Proceedings of 44th Kasetsart University Annual Conference**. pp. 553 – 561.

Chueaphothihack, V. 1999. **Nutrient and Aquatic Feeding,** Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand. Pp. 13 – 30.

Cabre-Pena, J.H., and H. Polimeni-Salinas. 1989. Variation in the growth and metamorphosis of pipens tadpoles under three different diets. **Aquaculture** 39: 25 – 32.

Crawshaw, G. 1993. **Amphibian Medicine**. In Fowler M (ed): Zoo and wild animal medicine (ed3).Philadelphia, Sauders, p 131-139.

Creswell, D.C. and A. Habibie. 1981. Studies on Snail meal as Protein Source for chicken. 2. Feeding value for laying hens. **Poultry Science** 60: 1861 – 1864.

Department of Fisheries. 1993. **Frog culture.** Reported conducted, Extension of Fisheries, Department of Fisheries. 22 p. (in Thai).

Favier, J.C. 1999. **Composition frog meat**, Ranajax, Import and Export. Ltd. http://www.ranajax.com. br/product.html. 15/ 10 / 2005.

Felix, N. and M, Sudharsan., 2004. Effect of glycine betaine, a feed attractant affecting growth and feed conversion of juvenile freshwater prawn *Macrobrachium rosenbergii*, **Department of Aquaculture, Fisheries College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University Thoothukudi, Tamil Nadu, India.** 193 – 197 pp.

Gomeez, K. A and A. A. Gomez. 1984. **Statistical Procedures for Agricultural Research** 2th Edition. John Willey, New york. 680 p.

Greg Lutz, C. and L. A, Jimmy., 1999. Bullfrog Culture, Southern Regional Aquaculture Center through Grant No, 94-38500-0045 from the Unites States Department of Agriculture, Cooperative States Research, **Education and Extension Service, SRAC Publication** No, 436.

Jilasack, X.1992. **Frog culture and Frog nursing for Trade**, V T S printing, Nonthaburi Thailand. Pp. 8 – 20. (inThai).

Hertrampf, J. W and F. Piedad-Pascual. 2000. **Handbook on Ingredients for Aquaculture Feeds**. Kluwer Academic Publishers, London. 573 pp.

Hinda, C., B, Juan and V, Erkki. 1998. Effect of FinnStim on growth and sea water adaptation of *Coho salmon*. **Aquaculture.** 168: 423 – 429.

Kanchanakhan, S. 1998. **An Ulcerative Disease of the Cultured Tiger Frog, *Rana tigrina*,** Aquatic Animal Health Research Institute Department of Fisheries, Bangkok, Thailand. 15 p.

Ketsada, M. 2005. Effect of Betaine on Growth performance and Nutrient Utilization in Juvenile Black Tiger shrimp (*Penaeus monodon,* Fabricius)**. The Proceedings of 44th Kasetsart University Annual Conference**. pp. 605 – 611.

Khamsivilay, L. and S. Phanousith., 2002. **Frog Culture in Earth pond by Using Difference Feed,** Living Aquatic Resource Research Center, National Agriculture and Forestry, Ministry of Agriculture and Forestry .pp. 10 – 15. (in Laos)**.**

Khamsivilay, L., S. Phanousith and B, Vongvichith. 2003. **Frog Breeding and Nursing From Natural and Semi artificial**, Living Aquatic Resource Research Center, National Agriculture and forestry Research Institute, Ministry of Agriculture and forestry. pp. 15-30. (in Laos).

Louis., A. H, Rechard, J. N. and P, James., 2001. Commercial Frog Farming, **Virginia Cooperative Extension, Publication**. 255 – 420 pp.

Lamlirtdesa, Th. 1988. **Frog culture in Thailand. Journal Aquaculture**. 41 (2): 101 – 115.

Méndez, E. JAOCS. 1998. Fatty acid composition of bullfrog, **Virginia Cooperative Extension Publication**, 110 – 150 pp.

Muirson, D. 2004. Liver fluke Disease and the Liver Fluke Snail, Department of Agriculture, **Government of western Australia ISSN** 0726-934X.

Marttinez, I.P., Herrez, M.P. and Alvalez, R. 1993. **Optimum Level of Dietary Protein for *Rana perezi* Seane larvae**. Aquaculture and Fisheries Management. 271-278 pp.

Millamena, O. M. 1994. Review of SEAFDEC/AQD fish nutrition and feed development researches. In Sandiago, C.B., R.M. Coloso, O. M. Millamena and L. G. Borlanggan(eds) **Feed for Small – Scale Aquaculture, Proceeding of the National Seminar – Workshop on Fish Nutrition and Feeds.** SEAFDDEC Aquaculture Development, Iloilo, thenPhilippines, pp. 52 – 63.

Millamena, O. M. Relicardo, M. C and Felicitas, P.P. 2002. **Essentials of Fish Nutrition, Feeds and Feeding of Tropical Aquatic Species**, SEAFDDEC Aquaculture Development, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines, p. 2 – 70.

Mokhamad, H. 2003, **Replacement of fish meal with Golden Apple Snail meal in Artificial Feed for Seabass (*Lares calcarifer, Bloch*).** Master Thesis, Faculty Fisheries, Kasetsart University, Bangkok. pp. 30 – 55.

National Research Council. 1993. **Nutrient Requirement of Fish.** National Academic Press, Washington.D.C. 115pp.

Namxongxay, A.1994. **Frog culture for Commerce trade**, Cooperative Frog culture, O S printing house, Bangkok, Thailand . pp.10-20.

Patterson, B. D., M. A. Rimmer., G. M. Meikle and G. L. Semeness. 2003. Physiological responses of the asian sea bass, *Latescalcarifer* to water quality deterioration during stimulated live transport: acidosis, red cell swelling, and level of ions and ammonia in plasma. **Aquaculture.** 218: 715 – 728.

Rechard, L. 2003. **Cattle Disease Focus Liver fluke Infection Fasciolosis, the meat and Live stock Commission is a Sponsor of NADIS** (National Animal Disease Information service, which is a network of 40 veterinary practices and 6 veterinary college monitoring disease in cattle, sheep, pig in the Ukhen.

Robison,D. G. 2004. **Safeguarding Intervention and Trade Compliance Officers Confiscate Giant African Snail in wisconsin**, United States Department of Agriculture, Animal and plant Health Inspection Service, APHIS 81-35-008.

Sativalakoon., S. 1994. **Hand book Frog culture for Trade,** O S printing house**,** Bangkok, Thailand. pp. 55 – 65.

Somsueb, P. M. Boonyaratpalin,. 2000. Optimal Protein and Energy Level for the Thai Native frog, *Rana rugulosa* weigmann, Feed Quality Control and Development Division, Department of Fisheries, **Aquaculture research** Bangkok, Thailand. 33-38 pp.

Sirois, M. 1995. **Veterinary Clinical Laboratory Procedures.** Mosby – Year Book Inc. St. Lois, Missouri. 328 pp.

Thongkongthay, T. 2004. **Reported Frog Processing and Frog Culture**, Faculty of Fisheries Latsmongkhon Technology Institute. 10-35 pp.

Thongoulay, K. 1977. **Experiment Frog culture**, Department of Fisheries, Annual Report 1966, Bangkok 50-81 pp

Vongvichith, B. L. Khamsivilay and X. Chounlamany 2002. **Frog Culture in Cages by Using Difference Feed,** Living Aquatic Resource Research Center, National Agriculture and Forestry, Ministry of Agriculture and Forestry. pp 19-30. (in Laos).

Wedemeyer, G. A. 1996. **Physiology of Fish in Intensive Culture System.** Chapman and Hall. United States of America. 232 pp.

Wilson, 1980. Preparation and evaluation of diets containing fish silage, cooked fish preserved with formic acid and low-temperature dried fish meal as protein sources for mirror carp (*Cyprinus carpio*). **Aquaculture,** 44: 27-40

Wilson, R.P and W. R. Poe. 1985. Apparent digestible protein and energy co efficiency of common feed ingredient for channel catfish. **Prog. Fish – Cult. 47: 152 – 159.**

World health, R. 2002. Disease Information, Related websites WHO. **http://www.who.int/ealth-topics/schisto.htm**. 20 / 10 / 2005

Xengtham, S. 1999. **Frog culture**, Theouphitack printing, Bangkok Thailand. pp 10–30. (in Thai).

APPENDIXS

Appendix Table 1 Air temperature in the morning and after noon, minimum and maximum water temperature in the morning water temperature in the after noon minimum and maximum (experiment 1)

No	Date	Air temperature		Water temperature			
				Morning		After noon	
		Morning	After noon	Min	Max	Min	Max
1	15/6/2005	26	28	24	25	26	27
2	16/6/2006	26.5	28.5	24	26	25	27
3	17/6/2007	27	29	25	27	26	28
4	17/6/2008	27	29	25	28	25	28
5	18/6/2009	26	27.5	24	26	25	27
6	19/6/2010	27	29	26	27.5	25	27
7	20/6/2011	26	28	24	26	25	26
8	20/6/2012	27	28.5	26	26	25	27
9	21/6/2013	27.5	29	25	27	26	28
10	22/6/2014	28.5	30	26	27	26	29
11	23/6/2015	27	29	25	27	26	28
12	23/6/2016	26.5	28.5	25	26	25	27
13	24/6/2017	27	29	25	26	26	28
14	25/6/2018	28	30	26	28	26	29
15	26/6/2019	28.5	29.5	26	27	26	28
16	26/6/2020	27	29	25	26	28	27
17	27/6/2021	28	29	26	26	26	28
18	28/6/2022	29	30	27	28	28	29
19	29/6/2023	30	31	28	29	28	29
20	29/6/2024	30.5	32	28	29	27	29
21	30/6/2025	30	31	27	28	28	30
22	1/7/2026	31	32	28	29	29	30
23	2/7/2027	30	31	28	29	29	30
24	2/7/2028	29	30	27	28	28	29
25	3/7/2029	29.5	30.5	28	29	29	30
26	4/7/2030	28	29	26	27	28	28
27	5/7/2031	29	30	26	28	28	29
28	5/7/2032	28.5	30.5	26	27	28	30

Appendix Table 2 Dissolved oxygen, pH, NO_2, NH_3, orthophosphate and total phosphorus concentration (experimental 1)

Treatment	Parameter						
	NO_2	NH_3	DO	pH	OrP	TP	BOD
The first time change water							
After change water							
T1	0.01	0.04	1.35	7	0.04	0.05	20
T2	0.03	0.05	1.4	6.8	0.08	0.085	23
T3	0.02	0.05	1.38	6.8	0.07	0.08	24
T4	0.02	0.05	1.39	6.9	0.06	0.074	22
Before change water							
T1	0.07	0.46	0.87	6.9	0.11	0.33	49
T2	0.1	0.59	0.75	6.5	0.35	0.65	0
T3	0.08	0.45	0.89	7	0.13	0.3	0
T4	0.06	0.42	0.9	7.1	0.21	0.63	0
The second time change water							
After change water							
T1	0.02	0.03	1.42	7	0.03	0.05	23
T2	0.02	0.01	1.5	6.8	0.04	0.06	28
T3	0.03	0.03	1.45	6.9	0.05	0.07	27
T4	0.03	0.05	1.38	7	0.03	0.04	30
Before change water							
T1	0.27	0.63	0.75	7.2	0.1	0.38	45
T2	0.1	0.4	0.83	7	0.39	0.82	51
T3	0.09	0.35	0.95	6.8	0.15	0.45	44
T4	0.06	0.24	0.99	6.5	0.23	0.61	54

Appendix Table 3 Air temperature in the morning and after noon, minimum and maximum water temperature in the morning, minimum and maximum water temperature in the after noon (experiment 2.1)

No	Date	Air temperature		Water temperature			
				morning		After noon	
		Morning	After noon	Min	Max	Min	Max
1	14/10/2006	25	27	25	26	24	26
2	15/10/2006	24	26	24	25	24	25
3	16/10/2006	26	28	25	26	26	27
4	17/10/2006	26	28	24	25	25	27
5	18/10/2006	27	29	26	27	27	28
6	19/10/2006	27.5	30	26	27	27	29
7	20/10/2006	28	30	27	28	28	29
8	21/10/2006	28	31	26	28	28	30
9	22/10/2006	27	29	26	27	26	28
10	23/10/2006	26	28	25	27	27	27
11	24/10/2006	25	27	24	26	25	26
12	25/10/2006	25	26	23	25	25	25
13	26/10/2006	26	27	24	25	25	26
14	27/10/2006	25	26	23	24	24	25
15	28/10/2006	24	26.5	22	24	24	25
16	29/10/2006	25	27	23	24	25	26
17	30/10/2006	25	27	23	26	24	26
18	31/10/2006	24	26	23	25	25	25
19	1/11/2006	24	26.5	22	24	24	25
20	2/11/2006	23	25	22	24	24	24
21	3/11/2006	24	26	22	23	24	25
22	4/11/2006	23	25	22	23	23	24
23	5/11/2006	24	26.5	23	24	24	25
24	6/11/2006	25	27.5	23	24	24	26
25	7/11/2006	24	26	23	24	24	25
26	8/11/2006	24	26	22	23	24	25
27	9/11/2006	23	25	22	23	24	25
28	10/11/2006	23	25	22	23	23	24

Appendix Table 4 Dissolved oxygen, pH, NO_2, NH_3, orthophosphate and total phosphorus concentration (experimental 2.1)

Treatment	Parameter						
	NO_2	NH_3	DO	pH	OrP	TP	BOD
The first time change water							
After change water							
T1	0.018	0.041	1.58	7	0.04	0.05	22
T2	0.032	0.056	1.45	7.2	0.07	0.07	25
T3	0.032	0.05	1.54	7.1	0.05	0.06	24
T4	0.032	0.055	1.45	7.2	0.06	0.06	21
Before change water							
T1	0.073	0.49	0.65	6.8	0.12	0.39	32
T2	0.096	0.54	0.58	7	0.37	0.43	37
T3	0.12	0.55	0.53	6.8	0.28	0.42	42
T4	0.069	0.46	0.7	6.5	0.19	0.32	32
The second time change water							
After change water							
T1	0.039	0.044	1.58	7.3	0.035	0.054	30
T2	0.037	0.041	1.62	7	0.05	0.063	30
T3	0.046	0.046	1.52	7.2	0.058	0.064	29
T4	0.034	0.035	1.7	6.7	0.037	0.055	32
Before change water							
T1	0.079	0.48	0.64	6.8	0.12	0.37	34
T2	0.1	0.59	0.54	7	0.3	0.41	40
T3	0.107	0.61	0.48	7.2	0.32	0.43	42
T4	0.07	0.46	0.68	6.5	0.2	0.35	36

Appendix Table 5 Air temperature in the morning and after noon, minimum and maximum water temperature in the morning, minimum and maximum water temperature in the after noon (experiment 2.2)

No	Date	Air temperature		Water temperature			
				Morning		After noon	
		Monin	Afternoon	Min	Max	Min	Max
1	15/10/2005	25	27	25	26	24	26
2	16/10/2005	24	26	24	25	24	25
3	17/10/2005	26	28	25	26	26	27
4	18/10/2005	26	28	24	25	25	27
5	19/10/2005	27	29	26	27	27	28
6	20/10/2005	27.5	30	26	27	27	29
7	21/10/2005	28	30	27	28	28	29
8	22/10/2005	28	31	26	28	28	30
9	23/10/2005	27	29	26	27	26	28
10	24/10/2005	26	28	25	27	27	27
11	25/10/2005	25	27	24	26	25	26
12	26/10/2005	25	26	23	25	25	25
13	27/10/2005	26	27	24	25	25	26
14	28/10/2005	25	26	23	24	24	25
15	29/10/2005	24	26.5	22	24	24	25
16	30/10/2005	25	27	23	24	25	26
17	31/10/2005	25	27	23	26	24	26
18	1/11/2005	24	26	23	25	25	25
19	2/11/2005	24	26.5	22	24	24	25
20	3/11/2005	23	25	22	24	24	24
21	4/11/2005	24	26	22	23	24	25
22	5/11/2005	23	25	22	23	23	24
23	6/11/2005	24	26.5	23	24	24	25
24	7/11/2005	25	27.5	23	24	24	26
25	8/11/2005	24	26	23	24	24	25
26	9/11/2005	24	26	22	23	24	25
27	10/11/2005	23	25	22	23	24	25
28	11/11/2005	23	25	22	23	23	24
29	12/11/2005	24	27	22	24	24	26
30	13/11/2005	24	27	23	24	25	26
31	14/11/2005	25	27.5	23	24	25	26
32	15/11/2005	24	26	23	24	24	25
33	16/11/2005	24	26	22	23	23	25
34	17/11/2005	23	25	22	23	23	24
35	18/11/2005	23	25	22	23	23	24
36	19/11/2005	24	26	22	23	24	25
37	20/11/2005	23	25	22	23	23	24
38	21/11/2005	24	26	22	23	24	25
39	22/11/2005	25	27	23	24	25	26
40	23/11/2005	25	27	23	24	24	26
41	24/11/2005	24	27	22	23	24	26
42	25/11/2005	24	26	22	23	24	25

Appendix Table 6 Dissolved oxygen, pH, NO_2, NH_3, orthophosphate and total phosphorus concentration (experimental 2.2)

Treatment	Parameter						
	NO_2	NH_3	DO	pH	OrP	TP	BOD
The first time change water							
After change water							
T1	0.025	0.043	1.6	6.8	0.04	0.057	18
T2	0.032	0.056	1.5	7.2	0.06	0.059	25
T3	0.034	0.057	1.52	7.1	0.06	0.063	27
T4	0.026	0.047	1.56	6.9	0.04	0.056	16
Before change water							
T1	0.09	0.57	0.6	7	0.18	0.37	43
T2	0.11	0.74	0.44	7.1	0.26	0.49	45
T3	0.133	0.99	0.4	7.2	0.28	0.5	46
T4	0.11	0.58	0.58	6.8	0.17	0.38	40
The second time change water							
After change water							
T1	0.037	0.04	1.65	6.9	0.037	0.05	22
T2	0.039	0.041	1.6	7	0.047	0.057	34
T3	0.042	0.044	1.56	7.1	0.051	0.06	35
T4	0.036	0.039	1.75	6.8	0.037	0.051	24
Before change water							
T1	0.093	0.6	0.4	6.8	0.19	0.39	33
T2	0.117	0.83	0.37	7.1	0.29	0.49	38
T3	0.12	0.93	0.32	7.2	0.32	0.51	40
T4	0.07	0.58	0.42	7	0.18	0.37	36