





Development of the Duckweed Holobiont Resource Values towards Thailand BCG Economy

Japanese side: Hokkaido U, Tohoku U, U Yamanashi, Kyoto U, Osaka U, National Institute for Environmental Studies (NIES), Saraya Co. Ltd.

Thai side: Kasetsart U, Mahidol U, Khon Kaen U, Chulalongkorn U, Nakhon Pathom Rajabhat U, Ramkhamhaeng U, NBT, BIOTEC, NANOTEC, NSTDA, ADGreen

Period of Project: October 2021 - September 2026 (5-year)





Previous Achievement (2022)

Research Grant: 2021-2022 6,559,100 THB, 400,000 JPY

Publications:

- 1. Saimee et al. 2021. IJSEM, 71 (11): 005106.
- 2. Yoksan et al. 2022. Int J Biol Macromol 203: 369-378.
- 3. Duangjarus et al. 2022. Foods, 11, 2348.

Conference:

- 1. The 14th Botanical Conference of Thailand, Khon Kaen University, 11-12 June 2022
- 2. International Conference of Genetics Society of Thaland (iGST2022), 21-22 June 2022.
- 3. Proceeding of the 11th International Conference on Environmental Engineering, Science and Management, Environmental Engineering Association of Thailand, 12 May 2022
- 4. The Proceedings of the 59th KU Annual Conference, 2002. 655-662





Project Monitoring Sheet I (2023)

(Annex 1-1, 1-2 Form 3-2)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
Overall Goal			
Research activities at the Duckweed Holobiont Resource & Research Center (DHbRC) are continued and further developed, and the research activities will be applied to biologica resources other than duckweed, so that the project's contribution to the Bio-Circular-Green (BCG) 2 economy will be recognized.	sequence data, and related information (at least 10 cases related to duckweed) (at least 5 cases other	>DHbRC and related organizations' materials >Interviews with DHbRC and related organizations	
	2. Number of technologies and cases applied socially by DHbRC and related organizations (at least 3)		
	3. Amount of R&D funding (budget) and project funding (budget) acquired by DHbRC and related organizations (3M THB)		2022 = 4,559,100 THB (KURDI), 400,000 JPY (Kurita Water and Environment Fund); 2023 = 300,000 THB (KURDI)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
Project Purpose			
A research development base is established for the development of duckweed industrial technology and its practical use which contributes to Thailand BCG economy.	1. Number of human resources trained (at least 20)		Training in Japan Long term: G3:4pp Short term: G1:2pp, G2:1pp, G3:3pp, G4-2:2pp, G4-3:1pp, G4-4:1pp, G5:1pp, G6:1pp
	2. Number of valuables developed using duckweed holobiont resources (at least 3)	>Project report >Monitoring report >Interview, survey and materials from related entities	
	3. Number of entities (universities, government agencies, companies) and a number of individual farmers for which DHbRC provided duckweed, associated microorganisms, DNA sequence data, and related information (at least XX entities, at least 3 individuals).		
	4. Number of technical manuals and proposals to promote the duckweed industry (at least XX)		Deleted OVI.4 in the PDM version 1.2



Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
Outputs			
1. Duckweed Holobiont Resource & Research Center (DHbRC) is established at Kasetsart University.	1. Establishment of DHbRC 2. Construction of a duckweed plant factory in DHbRC 3. Quantity of produced duckweed holobiont biomass to supply to the research groups (adequate amount for research requirement)	>Project report >Established DHbRC >DHbRC's homepage >Constructed duckweed plant factory in DHbRC >Interviews with DHbRC and research groups	DHbRC laboratory and office are established. JICA 1st batch equipment were delivered and operational (22 items). Procurement of JICA 2nd batch equipment is underway.
	4. Establishment of functions and services of DHbRC as a common laboratory		



Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
2. Duckweed holobiont collection is created	1. Number of preserved individual duckweeds, associated microorganisms, associated microbial communities, and their DNA sequence data (at least 20 plant specimens, 400 microbial strains, 20 microbial communities, 400 DNA sequence data)		
	2. Creation of a catalog of preserved organisms and relevant information		
	3. Number of academic papers (including peer-reviewed conference proceedings) published (at least 5)		1 Publication (AIMS Microbiol.), 2 Manuscript (in prep)

Biodiversity of Thai Duckweed Species





- Funding KURDI FF(KU)4.64, 65
- <u>Data</u>

G2

- 126+ DNA sequences
- Ecological data from 10 sites
- Morphology from 42 plants
- HPLC profiles for 4 species
- LC MS/MS QTOF data for 4 species

Research

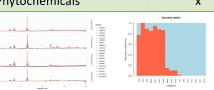
- 2 prepared MS
- 2 Bachelor's Thesis
- 1 PhD Student, 1 MS Student

Awards/Talks

- 1 National Conference Award
- 2 National Conference Talks
- 1 International Internship
- 1 International Conference

Research Plan

Topic	20	20	20	21	20)22	20	23
Collection	х	Х	х	х	х	х	х	Х
Morphology		Х	х	х	х			
Ecology		х	х	x				
Genetics	х	Х	Х	х	Х			
Phytochemicals						х	х	Х











Group PI









Senayai PhD Student MS Student

Jansantia



G2-2 Biodiversity of duckweed associated microbes



Assoc. Prof. Dr. Kannika Duangmal (PI; Actinobacteria) Dept. Microbiology, Fac. Science, KU

Assoc. Prof. Dr. Nantana Srisuk

Y2

The world first biobank of

duckweed holobionts



Assist, Prof. Dr. Chanita Boonmak (Bacteria) Dept. Microbiology, Fac. Science, KU



Assist. Prof. Dr. Pannida Khunnamwong (Yeast) Dept. Microbiology, Fac. Science, KU

Y4

Microbes	no. of isolates in collection	Species identification	PGP abilities
Bacteria	880	249	46
Actinobacteria	573	151	573
Yeast	252	252	252
Total	1,705	652	871

Culture of duckweedassociated microbiota Living microbial bioresources

Database of microbial community

Species identification and classification

PGP traits data

Υ3

Characterization of PGP abilities to enhance duckweeds biomass production, starch/protein production **Database of duckweed** associated microbes Ecology and taxonomy

Y5

Library of duckweedmicrobe holobionts Co-cultivation data for high-yield production of starch-rich and protein-rich duckweed biomass

Basement to establish duckweed-based BCG economy in Thailand

Current output 2023

Publication: 1

Submitted manuscript: 1

Manuscript: 1 Master students: 3

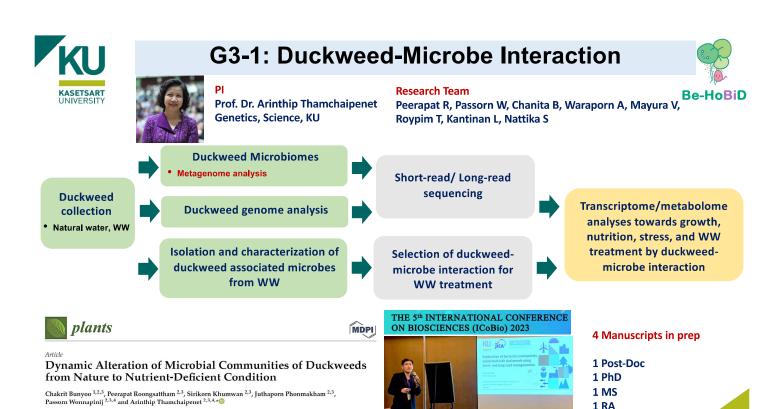
Output 2021-2022

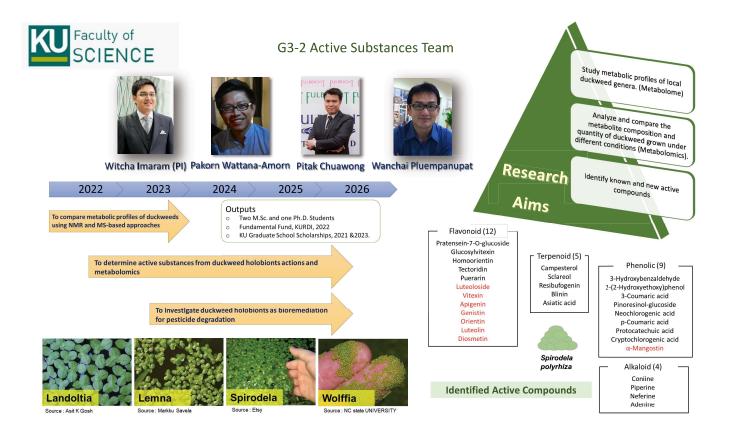
KU Graduate School Scholarship, 2022

Fundamental Fund, KURDI, 2022

Publication: 1 Master students: 3

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Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
3. Technology base is developed for function enhancement of the duckweed holobiont.	1. Number of potential duckweed holobionts for function enhancement and wastewater treatment (at least 10)	>Project report >Published academic papers related to the project	
	2. Number of omics 6 databases of duckweed and associated microorganisms at DHbRC (at least 5)		1 duckweed genome data with long-read and short-read data
	3. Number of analyses of duckweed and associated microorganisms' interaction (at least 2)		
	4. Number of technologies developed for improvement of duckweed holobionts for growth/stress tolerance (at least 2)		
	5. Number of active substances discovered/identified (at least 5)		
	6. Number of academic papers (including peer-reviewed conference proceedings) published (at least 10)		1 Publication (Plants), 3 Manuscript (in prep)





Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
<u>.</u>	· · · · · · · · · · · · · · · · · · ·		2 Thai petty patents
	2. Number of products of verified selected valuables at the laboratory level or bench plant scale (at least 3)	>Project report >Published academic journals related to the project	
	3. Number of academic papers (including peer-reviewed conference proceedings) published (at least 5)		4 Manuscript (in prep)

G4-1 BioFUEL: methane



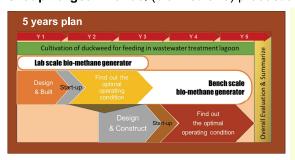








Group Target: Bio-fuel (bio-methane) production from Thai duckweed cultivated by wastewater



5) Thesis

Ardhana, M. Z. (2023). Evaluation of Methane Production Potential from Duckweed(Spirodela Polyrhiza) and Tapioca Starch Factory Wastewater as Co-Substrates [Master's Thesis, Khon Kaen University]

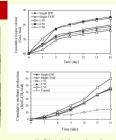


1) Established duckweed supply system at KKU (harvesting >200 g DW/week)



2) Set up Laboratory-scale reactors (ready for experiments)

3) Started-up the reactors (finished and continuing the experiments for the steady-state)



4) Obtaining the data of DW methane fermentation under different operation conditions

6) Manuscript - Choeisai P, Ardhana, M. Z., Thepubon T., Kubota K., 2023. Duckweed as a Co-Substrate for Methane Production from Tapioca Starch Factory Wastewater. (in prep)

7) Graduate students and scholarships for students

- Ms. Zhafirah Meuthia Ardhana: scholarship student from Indonesia AY 2021-2022 graduated master degree in time of 2 years, supported scholarship by Khon Kaen University (KKU) scholarship for ASEAN & GMS Countries' Personnel for Master student, 2021-2022 = 228,000 THB (2y), Supported research grant for master degree student 15,000 THB (2y)Graduated

 Ms. Sunisa Srisopa: scholarship for enhance productivity of Master degree student from Faculty of Engineering, KKU, 2023-2024 = 120,000 THB (2y) and Teaching Assistance Scholarship from Graduate School, KKU year 2023 = 30,000 THB (1y), Studying

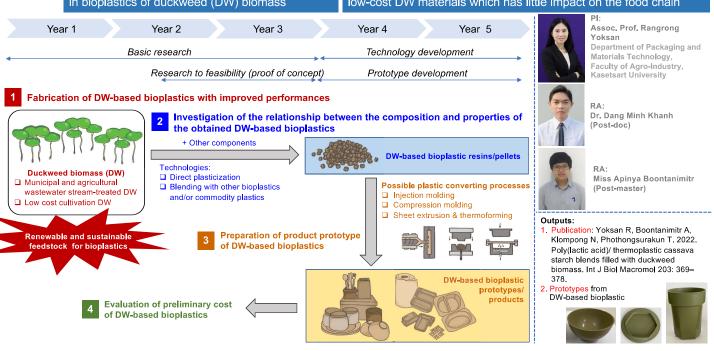
G4-2: Bioplastics from duckweed biomass

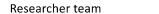


GOALS

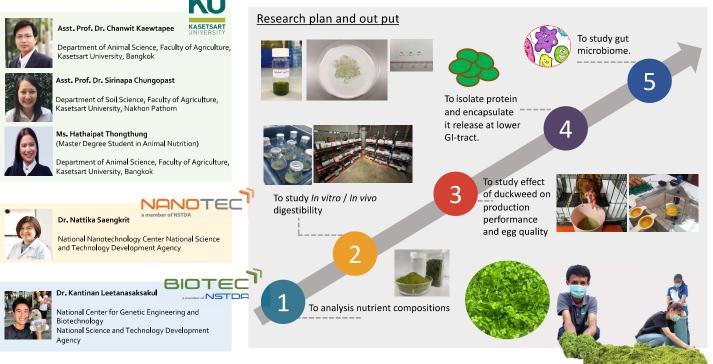
1. To add the value and expand the applications in bioplastics of duckweed (DW) biomass

2. To reduce the cost of bioplastics by replacing with renewable low-cost DW materials which has little impact on the food chain





Group: G4-3 Animal feed



Asst. Prof. Dr. Suvimol Charoensiddhi

(Dept. Food Science and Technology, Fac. Agro-industry, KU)- PI

Assoc. Prof. Dr. Massalin Nakphaichit

(Dept. Biotechnology, Fac. Agro-Industry, KU)- Member

Assoc. Prof. Dr. Wanwipa Vongsangnak (Dept. Zoology, Fac. Science, KU)- Member

Asst. Prof. Narissara Suratannon, M.D. (Dept. Pediatrics, Fac. Medicine, CU)- Member







1. Active-enriched components from duckweed leading to health benefits is developed.

2. Functional food applications are produced from duckweed active-enriched components.

Y1 Y2 Y5 2.4 Development of functional food 1.1 Collection and 1.3 Investigation of 1.4 & 1.5 Continue 2.2 Continue preparation of digestibility of active-2.1 Development of 2.3 Assessment enriched components in simulated human duckweed raw applications up-scale production process for activeof acute and materials subchronic toxicity 1.2 Development of enriched components of active-enriched production processes for duckweed activecomponents in 1.4 Assessment of 2.2 Study of physicochemical and an in vivo model health benefits enriched components functional properties 1.5 Characterization of major compositions

Key output: Digestibility and health benefits (anti-microbial activity and gut microbiome modulation) of duckweed and its protein and polysaccharide extracts. 2 Manuscripts, 1 oral presentation, and 1 Master student.

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Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
5. Low-carbon effect of the water purification system using duckweed holobiont is verified.	Energy saving rate of the entire system (Energy consumption lower than the Business-As-Usual (BAU))		
	2. Amount of green-house-gas (GHG) emission reduction (GHG emission lower than the BAU)	>Project report >Created database/handbook	
	3. Database/handbook for design and operation management of duckweed holobiont water purification system		
	4. Number of academic papers (including peer-reviewed conference proceedings) published (at least 5)		1 Book Chapter (in prep), 1 Manuscript (in prep), 2 Proceedings

G5: Duckweed-based wastewater treatment

PI: Chart Chiemchaisri, Dept. Environmental Eng. Faculty of Eng. Team: Wilai Chiemchaisri, 3 Postdoctoral researcher, 2 graduate students (1 Ph.D., 1 master)







2021 2022 2023 2024 2025 2026

> based WWT at bench or field scale operation at targeted WW source Dissemination of research results

Practical water purification of duckweed Database & guidelines of technology

Preparation

Evaluate duckweed base WWT performance at laboratory scale using livestock farm & food processing WW

Main findings (Oct22-Sep23)

- Appropriate duckweed species: Spirodela sp. for farm WW, Lemna sp. for food factory WW.
- Appropriate duckweed growth condition. 25-50% influent, 100% effluent, HRT 4-7 days, 75% initial duckweed coverage, 2-3 times/week harvesting
- WWT performance: Organic removal 60-90%, TN removals 30-60%
- Duckweed adaptation to survive higher WW conditions after appropriate microbial cultivation.

Research output (up to Sep 23)

- Book chapter: C. Chiemchaisri, W. Chiemchasri, L. Saksukol, C. Chandaravithoon, C. Witthayaphirom, V. Boonyaroj, T. Toyama, K. Mori, M. Morikawa, Duckweed-based waste stabilization ponds for wastewater treatment, In: Low-cost Water and Wastewater Treatment Systems, Elsevier (in press)
- Int. journal: W. Chiemchaisri, C. Chiemchaisri, L. Saksukol, T. Chanhaworn, K. Chuanprakop, T. Toyama, K. Mori, M. Morikawa Duckweed growth and pollutant removals in influent and effluent of condiments processing wastewater treatment system, Agriculture and Natural Resources journal (submitted)
- 2 conference proceeding disseminated at National Environmental Conference (May 2023)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
6. Support for duckweed production in farms and practical application of technology using duckweed are promoted.			
		>Project report	
	2. Number of support activities for duckweed production (at least 3)	>Dissemination and public relations activity reports >Created technical manuals and proposals	
	3. Number of created technical manuals and proposals of technology using duckweed (at least 3)		

Duckweed cultivation for farmers at Suphanburi











Be-HoBiD Output 2023

(Annex 2)

Publications:

- 1. Bunyoo C, Roongsattham P, Khumwan S, Phonmakham J, Wonnapinij P, **Thamchaipenet A.** 2022. Microbial communities of duckweeds in nature and in nutrient deficient condition. **Plants**. 11, 2915.
- 2. Kajadpai N, Angchuan J, Khunnamwong P, **Srisuk N**. 2023. Diversity of duckweed (Lemnaceae) associated yeasts and their plant growth promoting characteristics. **AIMS Microbiology.** 9(3): 486-517.

Manuscript (in prep):

- 1. Senayai, A., **Kraichak, E**. 2023. Species-specific chemical profiles of some duckweed species in Thailand, using HPLC and LC-QTOF-MS techniques. Phytochemistry (in prep).
- 2. Senayai, A. **Kraichak, E**. 2023. Genetic and morphological variation among populations of duckweed species found in Thailand. Plants. (in prep.).
- 3. Saimee, Y., **Boonmak, C**. and **Duangmal, K.** 2023 *Actinomycetospora lemnae* sp. nov., a novel actinobacterium isolated from *Lemna aequinoctialis* able to enhance duckweed growth. **Current Microbiology**. (in revision)
- 4. Duckweed associated bacteria as plant growth-promotor to enhance growth of *Spirodela polyrhiza* in wastewater effluent from a poultry farm (in prep)
- 5. Bunyoo C, Phonmakham J, **Thamchaipenet A**. 2023. High resolution profiling of microbial communities associated with duckweed, *Landoltia punctata* using full-length 16S rRNA sequencing. Phytobiomes J. (in prep).

(Annex 2)

Be-HoBiD Output 2023

Manuscript (in prep):

- 6. Bunyoo C, Thananusak R, Phonmakham J, **Thamchaipenet A**. 2023. Characterization of plastome sequences of the tiniest duckweed, *Wolffia globosa*, using long-read technology. BMC Genomics (in prep)
- 7. **Roongsattham P**, Khumwan S, **Boonmak C**, **Thamchaipenet A**. 2023. Plant growth promoting bacteria for promoting *Lemna aequinoctialis* growth (in prep)
- 8. Narsing Rao, MP, Thananusak, R, Bunyoo, C, Phonmakham, J, **Morikawa, M, Thamchaipenet, A** 2023. Microbial partnerships in duckweed ecosystems: A comprehensive review. Molecular Plant-Microbe Interaction (in prep).
- 9. Wacharakorn, P., **Peerapat, R., Imaram, W**. 2023. Metabolomic study of the effect of sucrose on *Spirodela polyrhiza* chemical profile (in prep)
- 10. **Choeisai P**, Ardhana, M. Z., Thepubon T., **Kubota K**. 2023. Duckweed as a co-substrate for methane production from tapioca starch factory wastewater (in prep)
- 11. **Yoksan R**, Boontanimitr A 2023. Effect of calcium carbonate on the performance of poly(butylene adipate-coterephthalate) filled with duckweed biomassEffect of calcium carbonate and titanium dioxide on properties of biodegradable polyesters filled with duckweed biomass. **Indust Crop Prod** (in revision)
- 12. Khanh DM, **Yoksan R, Matsumoto K, Morikawa M**. 2023. Duckweed biomass as alternative to cassava starch in manufacturing of TPS/PHBV/PBAT ternary blend (in prep).

Be-HoBiD Output 2023

(Annex 2)

Manuscript (in prep):

- 13. Thongthung, H., Chungopast, S., **Kaewtapee, C.** 2023. Chemical composition and In vitro protein digestibility of duckweed and feed ingredients. (in prep)
- 14. Dhamaratana S., Methacanon P., **Charoensiddhi S.** 2023. Chemical composition and in vitro digestibility of duckweed (*Wolffia globosa*) and its polysaccharide and protein fractions. Plant Foods for Human Nutrition (in prep)
- 15. **Charoensiddhi S.**, Natchaya D., Weerachai C., Chitsiri R., Roytrakul S. 2023. Synthesis and characterization of peptides derived from duckweed (*Wolffia globosa*) for enhancing antimicrobial properties. Food Control (in prep)
- 16. **Chiemchaisri C, Chiemchaisri W**, Saksukol L, Chandaravithoon C, Witthayaphirom C, Boonyaroj ., 2022. Duckweed based waste stabilization ponds for wastewater treatment. In "Low Cost Water and Wastewater Treatment Systems: Conventional and Recent Advances", Elsevier (in prep).
- 17. Saksukol L, **Chiemchaisri W, Chiemchaisri C.** 2023. Duckweed growth and pollutant removals in influent and effluent of food processing wastewater treatment. Thai Environmental Engineering Journal (in prep)



Be-HoBiD Output 2022 (Annex 3)

Conference:

- 1. Bunyoo C, Roongsattham P, Khumwan S, Phonmakham J, Thamchaipenet A 2023. Exploration of bacterial communities associated with duckweeds using short- and long-read metagenomics. The 5th ICoBio2023, Bogor, Indonesia (Oral presentation).
- 2. Thongthung H, Chungopast S, **Kaewtapee C**. 2022. Chemical composition and in vitro protein digestibility of duckweed. **The 11th National Animal Science Conference of Thailand**.
- 3. Development of functional ingredients from duckweed *Wolffia globosa* for gut microbiome modulation. "International Joint Seminar 2022" between Sejong University-Shinshu University-Kasetsart University.
- 4. Chandaravithoon C, Sutthasil N, **Chiemchaisri W, Chiemchaisri C.** 2023. Effect of organic pollutant concentrations on duckweed growth during piggery wastewater treatment using duckweed holobiont. **Proceeding of the 12th International Conference on Environmental Engineering, Science and Management**, 17-18 May 2023, Pattaya, Thailand.
- 5. Saksukol L, Chiemchaisri W, Chiemchaisri C. 2023. Duckweed growth and organic removals in influent and effluent of activated sludge wastewater treatment plant of food processing industry. Proceeding of the 12th International Conference on Environmental Engineering, Science and Management, 17-18 May 2023, Pattaya, Thailand.

No. of postgrad students & RA: 12 MS, 5 PhD, 5 Post-doc, 4 RA

Be-HoBiD Output 2023

Research grant:

Fundamental Fund, KURDI, KU
 SRU, KU
 SRU, KU
 Kurita Water and Environment Fund
 Research Grant for New Scholar, KURDI, KU
 2022 = 400,000 THB
 2023 = 300,000 THB



1. Canada-ASEAN scholarships and Educational Exchange for Development (SEED), EduCanada (Ph.D)

2. Post-Doc Scholarship, KU Reinventing U 2022 = **590,000** THB (1y)

3. KU Graduate School Scholarship 2021-2024 = **800,000** THB (2y) (4x)

4. KU Graduate School Scholarship
5. Post-Doc Fellowship, KU
6. Doctoral Scholarship from Fac. Engineering, KU
7. KKU scholarship for ASEAN & GMS Countries
8. Scholarship from Fac. Engineering, KKU
9. TA Scholarship, KKU
2022 = **300,000** THB (3y)
2021 = **228,000** THB (2y)
2023 = **120,000** THB (2y)
2023 = **30,000** THB (1y)

Awards: Good level academic oral presentation award, The 11th National Animal Science Conference of Thailand (2022)

(Annex 2)





Awards: The 11 national animal science conference of Thailand 2023



