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APPENDICES

APPENDIX A

PROXIMATE ANALYSIS (AOAC, 1995)

1. Protein analysis

1.1 Apparatus

- Kjeldahl flask
- Gerhardt Kjeldahl digestion unit
- Gerhardt vapodast 1

1.2 Reagent

- 50% NaOH
- 4% Boric acid
- Anhydrous sodium carbonate
- Bromocresol green
- Methyl red
- 95% Methanol
- Conc. Sulfuric acid
- Conc. Hydrochloric acid
- Catalysts (7 g K_2SO_4 + 0.8 g $CuSO_4.5H_2O$)
- Distilled water

1.3 Determination

1.3.1 Reagent preparation

- Sodiumhydroxide solution: 400 g of NaOH were dissolved in water and diluted to 1,000 ml

- Sodiumhydroxide solution 1 mol/l

- Sodiumhydroxide solution 0.1 mol/l

- Bromocresol green solution: 0.1 g of Bromocresol green were dissolved in the 100 ml of ethanol

- Methyl red solution: 0.1 g Methyl red was dissolved in 100 ml of ethanol

- Mixed indicator solution: 0.1 g of Bromocresol green and Methyl red was dissolved in 100 ml of ethanol

- Boric acid solution: 400 g of Boric acid was dissolved in approximately 6 L of distilled water then boil on hot plate, swirled till completely dissolving. The solution was adjusted to 9 L with hot distilled water. The solution was allowed to cool to the room temperature. Then the 100 ml of Bromocresol green solution and 70 ml of methyl red solution were added respectively. The solution was added to 10 L with distilled water and swirl to mix. Twenty five ml of Boric acid solution was pipetted in flask and then added with 100 ml of distilled water. The solution was titrated with 0.1 mol/l NaOH until the color changed to red-violet. Calculate the amount of used 1 mol/l NaOH for 10 L by criteria following:

ml of 1 mol/l NaOH = ml of mol/l NaOH

The amount of 1 mol/l NaOH was added into Boric acid solution and swirled well.

- Hydrochloric acid, 0.1 mol/l HCl, standardized

Eight point two ml of conc.HCl was pipetted and diluted with distilled water to 1,000 ml

Standardized: 5 g of anhydrous Na_2CO_3 was grinded, dried at 265 °C for 1 hr or at 200 °C for 2 hrs and allowed to cool in desiccator.

Point thirteen g of dried Na_2CO_3 was added into a flask and the 20 ml of distilled water was added. Five drops of indicator was mixed and then titrated with hydrochloric acid solution until color changed to pink. The amount of used HCl (A1) was recorded. The flask was removed into the water bath and boiled for 2-3 minutes. The flask was cooled to the room temperature (violet solution) then the solution was titrated with hydrochloric acid until the color changed to pink. The amount of used HCl (A2) was recorded. Calculate for concentration of HCl solution by the following equation.

$$\text{HCl mol/l} = \frac{2000 \times \text{exactly weight of } Na_2CO_3}{(A1+A2) \times \text{MW of } Na_2CO_3}$$

1.3.2 Analysis of protein

Two grams of sample was addedinto a digestion tube. Seven grams of catalyst and the 10-15 ml of conc.H_2SO_4 were added respectively. The digestion tube was transferred to the Kjeldahltherm at 420 °C until the solution was cleared then removed from the digester and allowed to cool. A flask containing 25 ml of 4% Boric acid and the digestion

tube were transferred into a distillator (Vapodest 1). The solution was titrated with standardized HCl until the solution turned to pink.

1.4 Calculation

Let

f = 6.25 for general factor

Then

$$\%N = \frac{14.001 \times [HCl] \times (\text{volume of HCl with sample} - \text{volume of HCl with blank}) \times 100}{\text{Weight of sample (mg)}}$$

% Protein = % N x f

2. Fat analysis

2.1 Apparatus

- Soxtherm automatic 8-11, Garhardt
- Desiccator

2.2 Determination

The extraction beaker was dried in hot air oven, preheating 100 °C for 1-2 hrs. The flask was cooled in a desiccator at room temperature and weighed. The process was repeated until the results of two successive weightings did not differ by more than 1 mg. The least weight of beaker (X) was record. The dried sample was grinded and weighed for approximately 2 g, wraped in a filter paper Whatman no. 1 and placed in a thimble. In the extraction beaker, the thimble was placed and 75 ml of petroleum ether was added. The extraction beaker was then connected with the soxtherm automatic. Switched the machine on and extract for 6 hrs. The thimble was removed from the extraction beaker

and the petroleum ether was evaporated to dryness at 120 °C for 1 hr. The beaker was cooled in the desiccator and weighed (Y)

2.3 Calculation

Let

W = Weight (g) of dried sample

Y = Weight (g) of the beaker and extracted sample

X = Weight (g) of the beaker

Then

$$\text{Total fat (\%)} = \frac{100\,(X-Y)}{W}$$

3. Ash analysis

3.1 Apparatus

- Porcelain crucible
- Furnace (Carbolite, model EML 11/2 serial no. 11/86/1468, Bandford, Sheffield, England)
- Desiccator
- Hot plate

3.2 Determination

A porcelain crucible was placed into the temperature-controlled furnace, preheated to 600 ± 20 °C for 1 hour then transferred directly to desiccator for cooling and weighed immediately. The crucible was re-ignited for 30 minutes; cool into the desiccator and weighed (W). This process was repeated until the results of two successive

weightings did not differ by more than 1 mg. Five grams (W1) of sample was added into the crucible. Then it was placed on the hot plate under a fume-hood and slowly increased the temperature until the smoking ceased. The crucible was placed inside the furnace at 600 ± 20 °C for 2-3 hours till the sample became thoroughly ash. Then the crucible was removed from the desiccator to cool and weighed (W2).

3.3 Calculation

Let

W = Weight (g) of porcelain crucible

W1 = Weight (g) of sample

W2 = Weight (g) of crucible and sample

Then

$$\text{Ash (\%)} = \frac{100\ (W2-W1)}{W1}$$

4. Fiber analysis

4.1 Apparatus

- Digestion apparatus: with condenser to fit 600 ml beaker
- Filtration apparatus: filter paper no.1 and no.42
- Gooch crucible of alundum crucible R-98
- Desiccator
- Oven
- Furnace (Carbolite, model EML 11/2 serial no. 11/86/1468, Bandford, Sheffield, England)

4.2 Determination

Two point five of dried samples were transferred into a 600 ml beaker. Two hundred ml of boiling 0.225 N sulfuric acid were added into the beaker and then placed on a digestion apparatus, allowed to heat till 30 minutes. The solution was then filtered through a filter paper no.1 using suction and then washed with the hot water (approximately 200 ml) until acid-free. The filter paper containing all insoluble matter was placed to the beaker. The 200 ml of boiling 0.313 N NaOH was added into the beaker and then the beaker was placed on the digestion apparatus, heated till 30 minutes. The solution was once filtrated through the filter paper no.42 using suction and then washed with hot water (approximately 200 ml) until base-free. The paper was washed with 10 ml 95% ethanol and then removed to a crucible and dried for 2 hrs in oven at 105-110 °C. The crucible was cooled in a desiccator, weighed and re-dried for 30 minutes until the results of two successive weighing did not more than 1 mg. The least weight (W1) was recorded. The crucible with containing fiber was ignited in the furnace 30 minute at 600 ± 15 °C, cooled in the desiccator and weighed. The crucible was re-ignited 30 minutes until the results of two successive weighing did not more than 1 mg. The least weight (W2) was weighed.

4.3 Calculation

Let

W = Weight (g) of sample

W1 = Weight (g) of crucible and insoluble matter

W2 = Weight (g) of crucible and ash

Then

$$\% \text{ Crude fiber} = \frac{100 (W1 - W2 - \text{filter paper weight})}{W}$$

5. Moisture content analysis

5.1 Apparatus

- Hot air oven

- Desiccator

- Porcelain crucible

5.2 Determination

Porcelain crucible was dried at 120 °C in hot air oven for 1 hr, cooled in desiccator and weighed (W1). Two grams of sample (W) were weighed in the crucible. The crucible was then dried in hot air oven 120 °C for 4 hrs. The crucible was cooled in desiccator and weighed (W2).

5.3 Calculation

Let

W = Weight (g) of sample

W1 = Weight (g) of crucible and sample

W2 = Weight (g) of crucible and dried sample

Then

$$\% \text{ Moisture content} = \frac{100 (W1 - W2)}{W}$$

APPENDIX B

DAVIDSON'S FIXATIVE

Davidson's fixative (Bell and Lightner, 1988) was made as such:

1. 330 ml 95% ethyl alcohol

2. 220 ml of 100% formalin (saturate aqueous solution of formaldehyde gas, 37-39% solution)

3. 115 ml of glacial acetic acid

4. 335 ml of distilled water

Store at room temperature

APPENDIX C

STATISTICAL ANALYSIS

Table 1C. Analysis of variance of proximate analysis in 4 experimental diets.

	Source	Sum of Squares	df	Mean Square	F	Sig.
Protein	Between Groups	0.844	3	0.281	2.608	0.189
	Within Groups	0.432	4	0.108		
	Total	1.276	7			
Lipid	Between Groups	2.607	3	0.869	150.494	0.000
	Within Groups	0.023	4	0.006		
	Total	2.630	7			
Ash	Between Groups	0.395	3	0.132	2.562	0.193
	Within Groups	0.205	4	0.051		
	Total	0.600	7			
Moisture	Between Groups	3.917	3	1.306	54.722	0.001
	Within Groups	0.095	4	0.024		
	Total	4.013	7			
Fiber	Between Groups	1.273	3	0.424	2.675	0.183
	Within Groups	0.634	4	0.159		
	Total	1.907	7			

Table 2C. Duncan multiple's range test of lipid content in 4 experimental diets.

Treatments	N	Subset for alpha = .05		
		a	b	c
B	2	8.0700		
A	2	8.1750		
D	2		8.4450	
C	2			9.5100
Sig.		0.239	1.0000	1.0000

Table 3C. Duncan's new multiple range test of moisture content in 4 experimental diets.

Treatments	N	Subset for alpha = .05	
		a	b
C	2	6.42	
D	2	6.6	
B	2		7.665
A	2		8.08
Sig.		0.309	0.055

Table 4C. Analysis of variance of body weight of *M. rosenbergii* in the period of 16 weeks.

	Source	Sum of Squares	df	Mean Square	F	Sig.
week4	Between Groups	0.278	3	0.093	2.640	0.049
	Within Groups	13.901	396	0.035		
	Total	14.179	399			
week8	Between Groups	0.711	3	0.237	0.221	0.882
	Within Groups	424.699	396	1.072		
	Total	425.410	399			
week12	Between Groups	58.318	3	19.439	2.886	0.036
	Within Groups	2667.712	396	6.737		
	Total	2726.030	399			
week16	Between Groups	92.540	3	30.847	1.672	0.172
	Within Groups	7305.707	396	18.449		
	Total	7398.248	399			

Table 5C. Duncan's new multiple range test of body weight of *M. rosenbergii* in the fourth week.

Treatments	N	Subset for alpha = .05	
		a	b
A	100	0.270	
B	100	0.308	0.308
C	100		0.329
D	100		0.338
Sig.		0.149	0.284

Table 6C. Duncan's new multiple range test of body weight of *M. rosenbergii* in the twelfth week.

Treatments	N	Subset for alpha = .05	
		a	b
C	100	4.506	
D	100	4.824	4.824
B	100		5.328
A	100		5.451
Sig.		0.386	0.108

Table 7C. Analysis of variance of body weight of *M. rosenbergii* in the twentieth week.

source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	656.636	3	218.879	7.307	0.000
Within Groups	30133.417	1006	29.954		
Total	30790.053	1009			

Table 8C. Duncan's new multiple range test of body weight of *M. rosenbergii* in the twentieth week.

Treatments	N	Subset for alpha = .05	
		a	b
C	239	10.722	
D	243	10.964	
A	273		12.101
B	255		12.699
Sig.		0.619	0.220

Table 9C. Analysis of variance of body length of *M. rosenbergii* in the period of 16 weeks.

	Source	Sum of Squares	df	Mean Square	F	Sig.
week4	Between Groups	2.415	3	0.805	3.489	0.016
	Within Groups	91.384	396	0.231		
	Total	93.799	399			
week8	Between Groups	0.815	3	0.272	0.487	0.692
	Within Groups	221.083	396	0.558		
	Total	221.898	399			
week12	Between Groups	11.005	3	3.668	4.517	0.004
	Within Groups	321.634	396	0.812		
	Total	332.639	399			
week16	Between Groups	6.256	3	2.085	1.841	0.139
	Within Groups	448.446	396	1.132		
	Total	454.702	399			

Table 10C. Duncan's new multiple range test of body length of *M. rosenbergii* in the fourth week.

Treatments	N	Subset for alpha = .05	
		a	b
A	100	2.532	
B	100	2.637	2.637
C	100		2.700
D	100		2.737
Sig.		0.123	0.167

Table 11C. Duncan's new multiple range test of body length of *M. rosenbergii* in the twelfth week.

Treatments	N	Subset for alpha = .05		
		a	b	c
C	100	5.680		
D	100	5.698	5.698	
B	100		5.946	5.946
A	100			6.071
Sig.		0.888	0.052	0.327

Table 12C. Analysis of variance of body length of *M. rosenbergii* in the twentieth week.

source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.870	3	11.957	8.771	0.000
Within Groups	1371.358	1006	1.363		
Total	1407.229	1009			

Table 13C. Duncan's new multiple range test of body length of *M. rosenbergii* in the twentieth week.

Treatments	N	Subset for alpha = .05	
		a	b
C	239	7.616	
D	243	7.745	
B	273		7.995
A	255		8.091
Sig.		0.217	0.354

Table 14C. Analysis of variance of body weight of male and female *M. rosenbergii* at the end of the experiment.

source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	823.850	7	117.693	3.935	0.000
Within Groups	29966.203	1002	29.906		
Total	30790.053	1009			

Table 15C. Duncan's new multiple range test of body weight of male and female *M. rosenbergii* at the end pf the experiment.

Treatments	N	Subset for alpha = .05	
		a	b
C, male	119	10.129	
D, male	126	10.429	
C, female	120	11.309	11.309
D, female	117	11.541	11.541
B, male	143		11.973
B, female	130		12.243
A, male	147		12.592
A, female	108		12.845
Sig.		0.062	0.051

Table 16C. Analysis of variance of body length of male *M. rosenbergii* at the end of the experiment.

source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	64.636	7	9.234	6.891	0.000
Within Groups	1342.592	1002	1.340		
Total	1407.229	1009			

Table 17C. Duncan's new multiple range test of body length of male and female *M. rosenbergii* at the end of the experiment.

Treatments	N	Subset for alpha = .05			
		a	b	c	d
C, male	119	7.408			
D, male	126	7.573	7.573		
C, female	120		7.823	7.823	
B, male	143		7.871	7.871	
D, female	117			7.930	
A, male	147			7.958	
B, female	130			8.130	8.130
A, female	108				8.272
Sig.		0.259	0.053	0.061	0.331

Table 18C. Analysis of variance of the extension of the first segment of the abdomen of male and female *M. rosenbergii* at the end of the experiment.

source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	291.768	7	41.681	8.052	0.000
Within Groups	5181.927	1001	5.177		
Total	5473.695	1008			

Table 19C. Duncan's new multiple range test of the extension of the first segment of the abdomen of male and female *M. rosenbergii* at the end of the experiment.

Treatments	N	Subset for alpha = .05			
		a	b	c	d
C, male	119	12.429			
D, male	126	12.760	12.760		
B, female	120		13.166	13.166	
B, male	143		13.306	13.306	
A, male	146			13.385	
D, female	117			13.408	
C, female	130				14.010
A, female	108				14.241
Sig.		0.252	0.072	0.452	0.424

Table 20C. Analysis of variance of the Propodus length of male and female of male and female *M. rosenbergii* at the end of the experiment.

source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29.463	7	4.209	7.246	0.000
Within Groups	582.039	1002	0.581		
Total	611.502	1009			

Table 21C. Duncan's new multiple range test of Propodus length of male and female of male and female *M. rosenbergii* at the end of the experiment.

Treatments	N	Subset for alpha = .05		
		a	b	c
C, female	120	2.254		
D, male	126	2.289		
D, female	117	2.343	2.343	
C, male	119	2.346	2.346	
A, female	108		2.531	2.531
B, female	130			2.594
B, male	143			2.671
A, male	147			2.702
Sig.		0.392	0.064	0.107

Table 22C. Analysis of variance of the carpus length of male and female of male and female *M. rosenbergii* at the end of the experiment.

source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.787	7	2.112	6.831	0.000
Within Groups	309.854	1002	0.309		
Total	324.641	1009			

Table 23C. Duncan's new multiple range test of carpus length of male and female of male and female *M. rosenbergii* at the end of the experiment.

Treatments	N	Subset for alpha = .05		
		a	b	c
D, male	126	1.414		
C, male	119	1.441		
C, female	120	1.480		
D, female	117	1.526	1.526	
A, female	108		1.627	1.627
B, male	143		1.653	1.653
B, female	130			1.687
A, male	147			1.765
Sig.		0.148	0.089	0.073

Table 24C. Analysis of variance of the merus and ichium length of male and female of male and female *M. rosenbergii* at the end of the experiment.

source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	107.366	7	15.338	12.995	0.000
Within Groups	1182.663	1002	1.180		
Total	1290.030	1009			

Table 25C. Duncan's new multiple range test of merus and ichium length of male and female of male and female *M. rosenbergii* at the end of the experiment.

Treatments	N	Subset for alpha = .05		
		a	b	c
C, female	120	1.921		
D, female	117	2.012	2.012	
D, male	126	2.190	2.190	
A, female	108	2.196	2.196	
C, male	119		2.245	
B, female	130		2.248	
B, male	143			2.769
A, male	147			2.863
Sig.		0.067	0.129	0.497

Table 26C. Analysis of variance of the total claw length of male and female of male and female *M. rosenbergii* at the end of the experiment.

source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	344.497	7	49.214	9.176	0.000
Within Groups	5374.007	1002	5.363		
Total	5718.505	1009			

Table 27C. Duncan's new multiple range test of total claw length of male and female of male and female *M. rosenbergii* at the end of the experiment.

Treatments	N	Subset for alpha = .05				
		a	b	c	d	e
C, female	120	5.655				
D, female	117	5.881	5.881			
D, male	126	5.893	5.893			
C, male	119	6.032	6.032	6.032		
A, female	108		6.355	6.355		
B, female	130			6.528	6.528	
B, male	143				7.093	7.093
A, male	147					7.329
Sig.		0.246	0.143	0.110	0.054	0.420

Table 28C. Analysis of variance of the growth rate (weight basis) of *M. rosenbergii* in the period of 20 weeks.

	Source	Sum of Squares	df	Mean Square	F	Sig.
week4	Between Groups	8E-06	3	2.66667E-06	0.234	0.871
	Within Groups	0.000182	16	0.000011375		
	Total	0.00019	19			
week8	Between Groups	7.615E-05	3	2.53833E-05	0.076	0.972
	Within Groups	0.0053196	16	0.000332475		
	Total	0.00539575	19			
week12	Between Groups	0.0028314	3	0.0009438	0.470	0.707
	Within Groups	0.0321108	16	0.002006925		
	Total	0.0349422	19			
week16	Between Groups	0.0006844	3	0.000228133	0.094	0.962
	Within Groups	0.0387548	16	0.002422175		
	Total	0.0394392	19			
week20	Between Groups	0.0031828	3	0.001060933	0.614	0.616
	Within Groups	0.0276664	16	0.00172915		
	Total	0.0308492	19			

Table 29C. Analysis of variance of the growth rate (length basis) of *M. rosenbergii* in the period of 20 weeks.

	Source	Sum of Squares	df	Mean Square	F	Sig.
week 4	Between Groups	0.015	3	0.005	0.752	0.537
	Within Groups	0.109	16	0.007		
	Total	0.124	19			
week 8	Between Groups	0.030	3	0.010	1.222	0.334
	Within Groups	0.130	16	0.008		
	Total	0.160	19			
week 12	Between Groups	0.037	3	0.012	0.917	0.455
	Within Groups	0.215	16	0.013		
	Total	0.251	19			
week 16	Between Groups	0.005	3	0.002	0.200	0.895
	Within Groups	0.137	16	0.009		
	Total	0.142	19			
week 20	Between Groups	0.033	3	0.011	1.581	0.233
	Within Groups	0.112	16	0.007		
	Total	0.145	19			

Table 30C. Chi-square analysis of sex ratio *M. rosenbergii* at harvest.

	A	B	C	D
Chi-Square[a,b,c,d]	5.965	1.664	0.004	0.004
df	1	1	1	1
Asymp. Sig.	0.015	0.197	0.948	0.950

[a]0 cells (.0%) have expected frequencies less than 5.
The minimum expected cell frequency is 127.5.
[b]0 cells (.0%) have expected frequencies less than 5.
The minimum expected cell frequency is 132.5.
[c]0 cells (.0%) have expected frequencies less than 5.
The minimum expected cell frequency is 119.5.
[d]0 cells (.0%) have expected frequencies less than 5.
The minimum expected cell frequency is 125.5.

Table 31C. Analysis of variance of total weight *M. rosenbergii* in the period of 20 weeks.

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	87950.061	3	29316.687	1.281	0.315
Within Groups	366124.505	16	22882.782		
Total	454074.566	19			

Table 32C. Analysis of variance of percent survival *M. rosenbergii* in the period of 20 weeks.

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	139.800	3	46.600	0.140	0.935
Within Groups	5335.200	16	333.450		
Total	5475.000	19			

Table 33C. Analysis of variance of the number of ovigerous female *M. rosenbergii* in the period of 20 weeks.

source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32.950	3	10.983	4.625	0.016
Within Groups	38.000	16	2.375		
Total	70.950	19			

Table 34C. Duncan's new multiple range test of the number of ovigerous female *M. rosenbergii* in the period of 20 weeks.

Treatments	N	Subset for alpha = .05		
		a	b	c
D	5	0.600		
C	5	0.800	0.800	
A	5		2.800	2.800
B	5			3.600
Sig.		0.840	0.057	0.424

BIOGRAPHY

Miss Nipaporn Chokchaikasemsuk was born on March 22, 1979 in Saraburi Province. She graduated with a Bachelor degree of Science (Biotechnology) from Mahidol University in 1999. She studied for the Master degree of Science (Biotechnology) at Faculty of Science, Chulalongkorn University since 2003.