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APPENDICES

APPENDIX A

Ash of animal feed

Apparatus

-Furnace muffle

-Porcelain crucible

-Hot plate

Method

Porcelain crucible was dried in an oven at 105 ^{o}C for 2 hr and transfered directly to a desiccator until it was cool and then weighed immediately. The 2 g of dry sample was put in the crucible which was placed on a hot plate, in a hood until it was smokeless. It was placed in a furnace muffle heated at 600 ^{o}C and held at this temperature for 3 hr. The crucible was transfered to a desiccator until it was cool and weighed again.

% Ash = (weight of ash (g) x 100)/ weight of sample(g)

Crude fat in animal feed

Apparatus

-Soxtherm Automatic model S-11, Gerhardt, Germany

Reagent

-Petroleum ether (AR grade) was purchased from Mallinkordt, USA.

Method

Soxtherm beaker was dried in an oven at 130°C for 3 hr and transfered to a desiccator until it was cool at room temperature. Then it was weighed. Sample (2 g) was wrapped with 2 pieces of filter and put in the thimble that was in the beaker containing 80 ml of petroleum ether. Beaker was attached to Soxtherm to extract fat at 150°C, for 4-6 hr, controlled by heated silicone oil. Afterwards, petroleum ether was evaporated to dryness. Then beaker was dried in an oven at 120°C for 1 hr and left in a desiccator. Cool beaker was weighed and fat content was calculated.

% Fat = (weight of fat (g) x100)/ weight of sample (g)

Moisture in animal feed

Apparatus

- Sartorius Thermo Control model YTE01L, Germany

Method

Sample (2 g) was put on a dry tray with no moisture in moisture analyser at 130 °C. It was recorded when moisture in sample varied 0.1 % in 50 sec.

Crude Protein in animal feed

Apparatus

-Gerhardt Kjeldatherm Digestion Unit, Germany

-Gerhardt Vapodest1, Germany

Reagent

1. Sulfuric acid (AR), BDH, England
2. Sodium hydroxide (AR), Eka Nobel, Sweden
3. Boric acid (AR), Mreck, USA
4. Catalyst (Kjel-tab) was contained 3.5 g K_2SO_4 and 0.0035 g Se Tecator, Sweden

5. Indicator was contained 0.625 g of methyl red and 0.480 g of methylene blue which dissolved in ethyl alcohol (50 ml, 95% V/V).

Method

Sample (2g) in filter paper was put in a digestion tube with a size of 250 ml, added sulfuric acid (20 ml, conc.) and 1 Kjel-tab. It was placed in a digestion unit which is composed of a vaccumn hood and the system was preheated at 200°C for 20 min. Then the temperature of heating system was increased 20°C for every 20 min until it was at 380°C. After digestion, the solution was left at room temperature. The solution was added H_2O (90 ml). It was distilled with a solution of sodium hydroxide (70 ml, 50% V/V). Ammonia was collected in boric acid (50 ml, 4% w/V) and added 3-4 drops of indicator. It was titrated with sulfuric acid that was accurately prepared with known (0.5 N). The volume of sulfuric acid used in titrated was recorded and ammonia concentration was accordingly calculated.

$$\% \text{ protein} = (AxBx6.25x1.4)/C$$

A= normality of sulfuric acid used to titration

B= ml of sulfuric acid used to titration

C= weight of sample (g)

Crude fiber in animal feed

Apparatus

-Crude fiber digestion model RF-16/6 Gerhardt, Germany

Reagent

1. Sulfuric acid (AR), BDH, England
2. Sodium hydroxide(AR), Eka Nobel, Sweden
3. Ethyl alcohol, Thai victory, Thailand

Method

A crucible and filter Whatman no.41 were dried in an oven at 105°C for 2 hr, and transferred to a desiccator. Then they were weighed when they were cool. The sample with no fat, was accurately weighed and put in a beaker (500 ml). Then it was added sulfuric acid solution (200 ml, 0.255 N), digested and heated for 30 min. During digestion, the level of sulfuric acid solution was maintained constantly. Until the solution was homogenous, it was filtered through whatman no. 41 and precipitate was washed on a filter with H_2O in order to eliminate acid. The precipitate on a filter was put in the same beaker and added sodium hydroxide solution (200 ml, 0.313 N), which was then digested for another 30 min. The solution was filtered through the same filter and washed with H_2O in order to neutralize basic condition. Afterward, The

precipitate was washed with ethyl alcohol (300 ml, 95 % V/V). That filter paper containing precipitate was dried in an oven at 100°C for 2 hr and transferred directly to a desiccator. A cool filter paper weighed. A crucible, which had the filter and precipitate, was placed in furnace muffle and heated at 600°C for 3 hr. Then it was left to be cool and weighed.

$$\%\text{fiber} = \frac{(\text{weight of filter+percipitate-weight of filter-weight of ash}) \times 100}{\text{weight of sample (g)}}$$

APPENDIX B

CALCULATION METHOD

Calculation method, which was used to determine fatty acid concentration of sample is an internal standard.

Principle

The internal standard used in quantitation must also be resolved from all the components present, and should, ideally, be eluted somewhere near the middle of the mixture. The internal standard method uses the ratios of peak area to convert peak areas to concentrations. The ratios of peak areas remain unchanged although it has any variation during preparative process such as losses sample, and inaccurate injection volume. Any variation in conditions affects both analyte and internal standard alike (Willett, 1991).

Once a suitable internal standard has been chosen, this is probably the most reliable method available for quantitative analysis. In the present study, nonadecanoic acid (C 19:0) was used as an internal standard in fatty acid analysis.

Calibration of each component of interest (i)

$$RF_i = (CC_i/Area_i)x(Area_{Is}/CC_{Is})$$

where:

RF_i = Response factor for component (i)

$Area_i$ = Area or height of component (i)

$Area_{Is}$ = Area of internal standard peak

CC_{Is} = Amount of internal standard used in the calibration sample

CC_i = Amount of component (i) in the calibration sample

Response factor of each component is used to calculate the concentration of each component

Calculation of each component of interest (i)

$$Conc_i = (IS/SA) \times ((RF_i/Area_i)/(RF_{Is}/Area_{Is})) \times XF$$

where :

$Conc_i$ = Amount of component i in the sample.

IS = Amount of the internal standard added to the samples. Unit of measurement must be the same as those used in measuring the sample amount.

SA = Amount of sample material measured.

RF_i = Response factor for component (i) calculated in the calibration run.

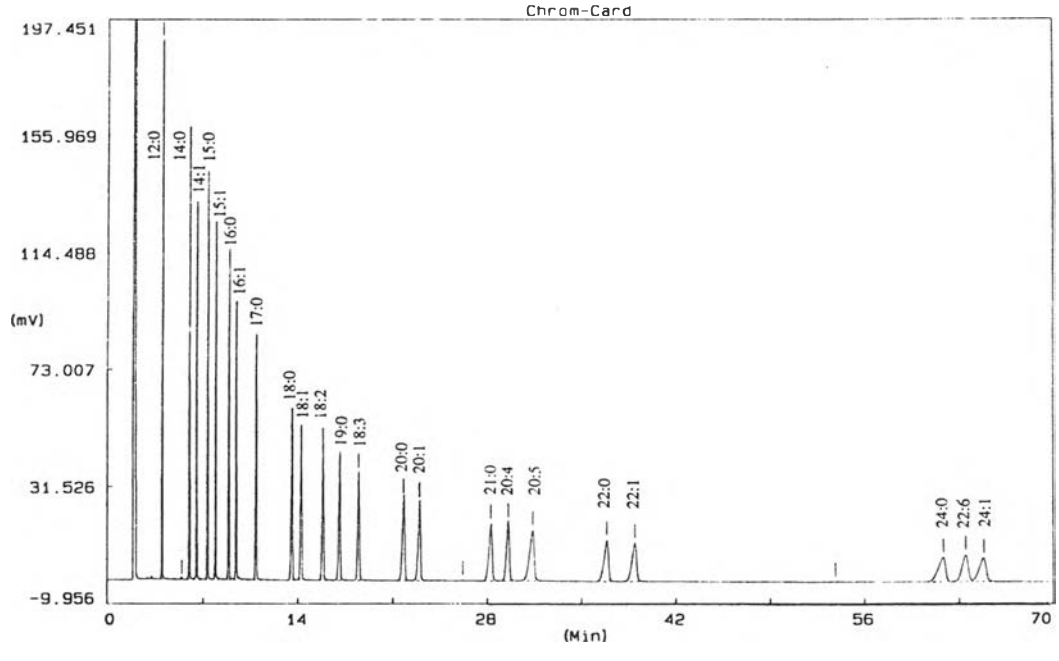
$Area_i$ = Area or height of component(i) in the analysis run.

RF_{Is} = Response factor of the internal standard by definition is 1

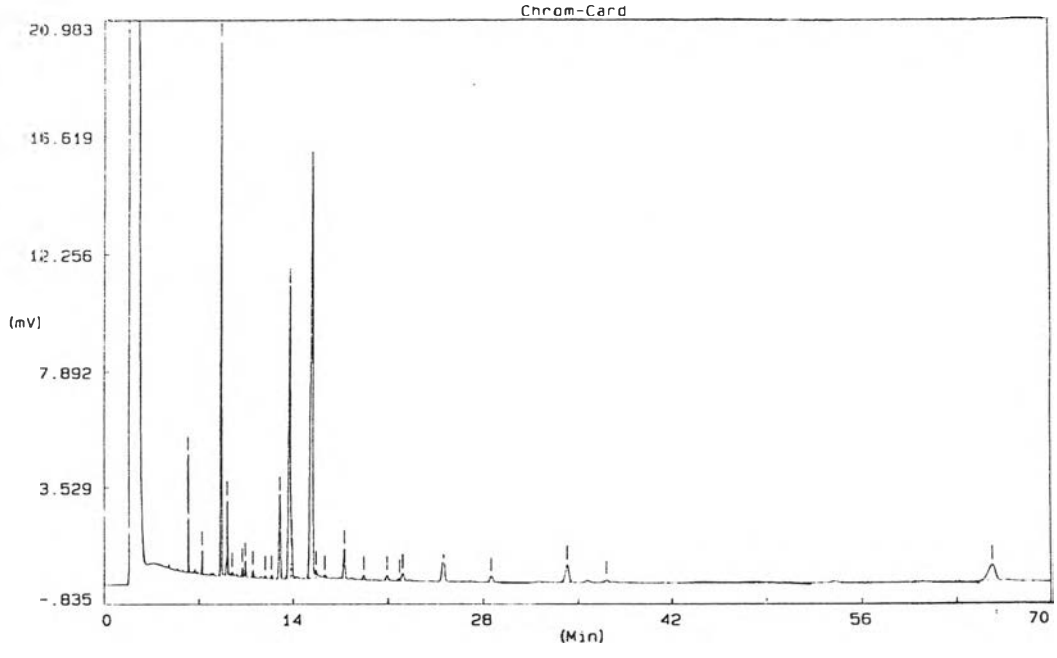
$Area_{Is}$ = Area of the internal standard peak in the sample

XF = Scaling factor (multiplier) which may be used as a convertion factor. If the $Conc_i$ is wanted in percentage, XF must be equal to 100, otherwise its use is optional

1. Typical chromatogram of reference standard.



2. Typical chromatogram of sample.



APPENDIX C

1. Statistical analysis of comparison on the final weight of postlarvae fed 7 diets.

General Linear Models Procedure

Class Level Information

Class	Levels	Values
TREAT	7	1 2 3 4 5 6 7

Number of observations in data set = 1319

General Linear Models Procedure

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.34480938	0.05746823	5.07	0.0001
Error	1312	14.86140821	0.01132729		
Corrected Total	1318	15.20621759			

R-Square	C.V.	Root MSE	WEIGHTMean
0.022676	52.40242	0.106430	0.20310083

Dependent Variable: WEIGHT

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	6	0.34480938	0.05746823	5.07	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	6	0.34480938	0.05746823	5.07	0.0001

Duncan's Multiple Range Test for variable: WEIGHT

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate Alpha= 0.05 df= 1312 MSE= 0.011327

WARNING: Cell sizes are not equal. Harmonic Mean of cell sizes= 185.4414

Number of Means	2	3	4	5	6	7
Critical Range	.0220	.0231	.0238	.0244	.0248	.0252

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	TREAT
	A		0.2270	202	6
	A				
B	A		0.2196	196	7
B	A				
B	A	C	0.2104	187	4
B		C			
B	D	C	0.1989	200	3
	D	C			
	D	C	0.1955	197	2
	D				
	D		0.1849	138	1
	D				
	D		0.1802	199	5

2. Statistical analysis of comparison on the percent survival of postlarvae fed 7 diets.

General Linear Models Procedure

Class Level Information

Class	Levels	Values
TREAT	7	1 2 3 4 5 6 7

Number of observations in data set = 20

General Linear Models Procedure

Dependent Variable: SURVIVAL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	145.2094967	24.2015828	1.58	0.2286
Error	13	198.6385833	15.2798910		
Corrected Total	19	343.8480800			

R-Square	C.V.	Root MSE	SUR Mean
0.422307	4.310518	3.908950	90.6840000

Dependent Variable: SUR

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	6	145.2094967	24.2015828	1.58	0.2286
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	6	145.2094967	24.2015828	1.58	0.2286

3. Statistical analysis of comparison on CMI of postlarvae fed 7 diets.

General Linear Models Procedure

Class Level Information

Class	Levels	Values
TREAT	7	1 2 3 4 5 6 7

Number of observations in data set = 14

General Linear Models Procedure

Dependent Variable: CMI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	656.1947464	109.3657911	4.38	0.0371
Error	7	174.8534625	24.9790661		
Corrected Total	13	831.0482089			

R-Square	C.V.	Root MSE	CMI Mean
0.789599	5.563672	4.997906	89.8310714

Dependent Variable: CMI

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	6	656.1947464	109.3657911	4.38	0.0371
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	6	656.1947464	109.3657911	4.38	0.0371

Duncan's Multiple Range Test for variable: CMI

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate Alpha= 0.05 df= 7 MSE= 24.97907

Number of Means	2	3	4	5	6	7
Critical Range	11.81	12.28	12.54	12.66	12.74	12.77

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TREAT
	A	103.770	2	5
	A			
B	A	92.930	2	3
B				
B	C	89.130	2	2
B	C			
B	C	88.963	2	6
	C			
	C	88.245	2	4
	C			
	C	86.300	2	1
	C			
	C	79.420	2	7

BIOGRAPHY

Miss Raweewan Suwanich was born on 23 July, 1969 at Nakorn Pathom Province. She was graduated her B.Sc. in General Science (Chemistry-Biology) from Prince of Songkla University in 1990. She had enrolled her study at Chulalongkorn University for Master Degree of Biotechnology in 1993.