CHAPTER 4

RESULTS

4.1 Cultivation of *Spirulina platensis*

4.1.1 Laboratory culture of *S. platensis*

Biomass production (g L^{-1}) of *S. platensis* cultured in different media, modified Zm, Kw and Sw, were measured, as dry weight, and compared among one another. Water quality from each of those media used, as culturing media filtrate are summarized and shown in (Appendix B. Table 8) and the progress of parameter changes are illustrated in Figure 22. Optimum N : P ratio and biomass production (dry weight) for the cultivation of *S. platensis* in Kw and Sw media were compared, and the results are also shown in (Appendix B. Table 8). The results indicated that the initial N : P ratio of modified Zm medium was 6.9 : 1 and changed to 1.2 : 1 at 15 days cultivation. The N : P ratio of Kw and Sw media ranged between 6: 1-6.2 : 1 and 8.2 : 1-8.3 : 1, and changed to 0.4 : 1-0.5 : 1 and 6.92 : 1-6.94 : 1, respectively, at 15 days. Each culturing bottle was measured for initial OD of 0.30 (OD_{560nm} = 0.30) as the initial biomass production of 0.30 g L^{-1}. After 15 days of cultivation, the biomass production has changed to range from 0.01 to 0.51 g L^{-1}.

According to the cultivation of *S. platensis* in laboratory of the present study, it was determined that the highest algal biomass production was obtained at day 10 of the cultures in 5%Sw (0.90 g L^{-1} and N : P = 6 : 1) which was higher than the cultures in 10-100%Kw media and 10-100%Sw media (Figure 22).

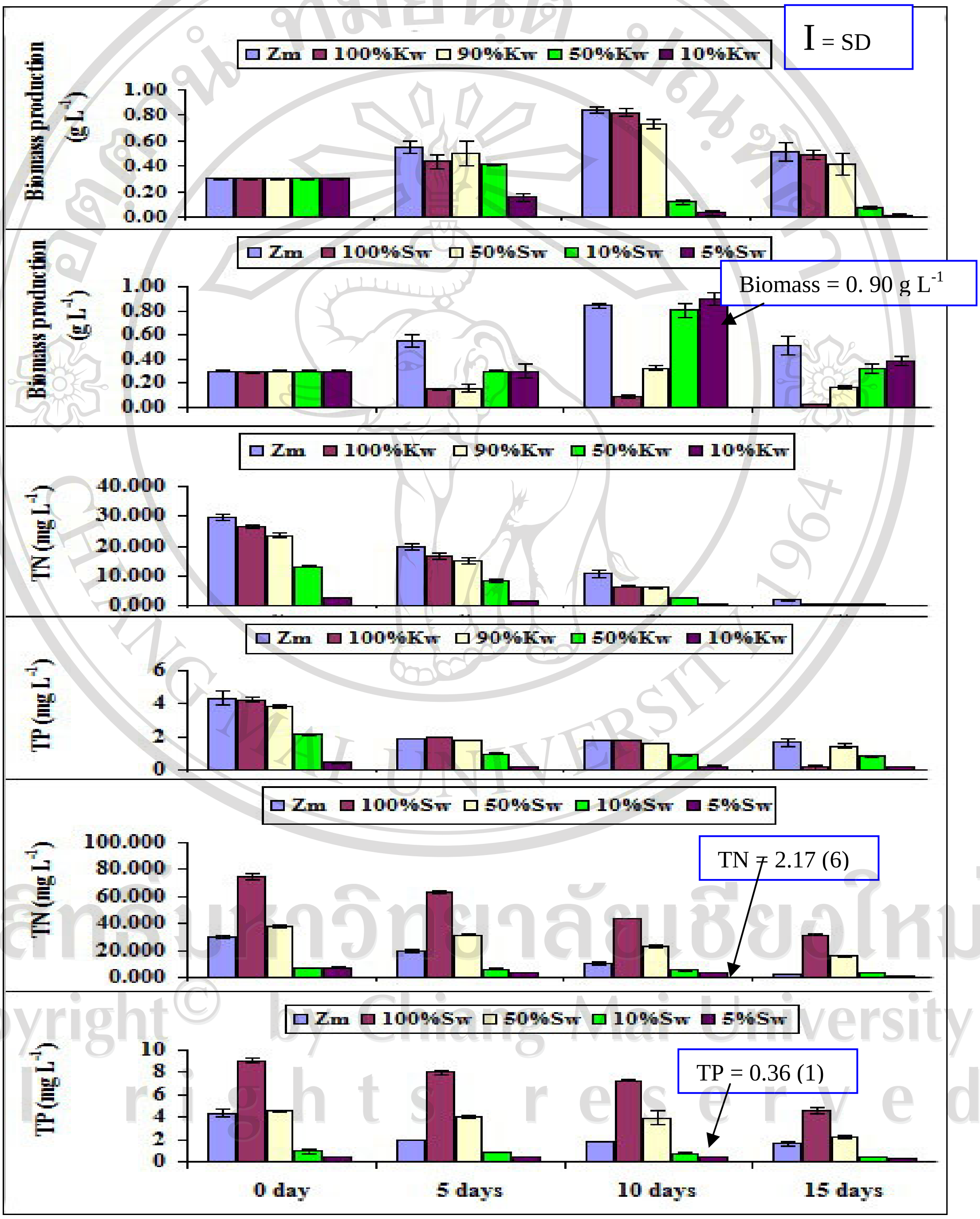

Figure 22 Changes of *S. platensis* biomass production (dry weight), TN and TP of modified Zm, Kw and Sw in laboratory culture for 15 days period

4.1.2 Outdoor mass culture of *S. platensis* in the experimental tanks

S. platensis from stock prepared in Kw medium were cultured in the 5 culturing media selected from laboratory culture including modified Zm, 90% and 100%Kw, and 5% and 10%Sw media. Then the biomass production (dry weight), production variable cost, β-carotene, C-phycocyanin and γ- linoleic acid, nutritional values and phytoremediation of wastewater by *S. platensis* were measured and compared among all the media.

4.1.2.1 The cultivation of *S. platensis*, using modified Zm, Kw and Sw media as culturing media, are summarized in (Appendix B. Table 9) and the progress of the changes is illustrated in Figure 23. The figures indicated that, for 10-day cultivation, the TN and TP in 100%Kw, 90%Kw, 10%Sw and 5%Sw media had decreased compared to that in the modified Zm, while the TN and TP in 10%Sw and 5%Sw media showed the values close to that of the modified Zm the 10–day (water of evaporation level 3 cm/ week). The biomass production obtained from 5%Sw medium was 0.90 g L^{-1} with the optimum N : P ratio of 6.3 : 1 which are higher than those in 10%Sw medium (0.85 g L^{-1} and N : P ratio of 6.4 : 1). The biomass and N : P ratio, the 10-day cultivation, measured in modified Zm, 100% and 90%Kw media were 0.84 g L^{-1} and 6.6 : 1, 0.82 g L^{-1} and 3.7 : 1, and 0.74 g L^{-1} and 3.7 : 1, respectively, with $p<0.05$ (Appendix B. Table 9). The pH values in 100% and 90%Kw media were higher than that in the modified Zm medium, whereas the values in 10% and 5%Sw media were lower than that in the modified Zm for all time cultivation at 0, 5, 10 and 15 days (Figure 23).

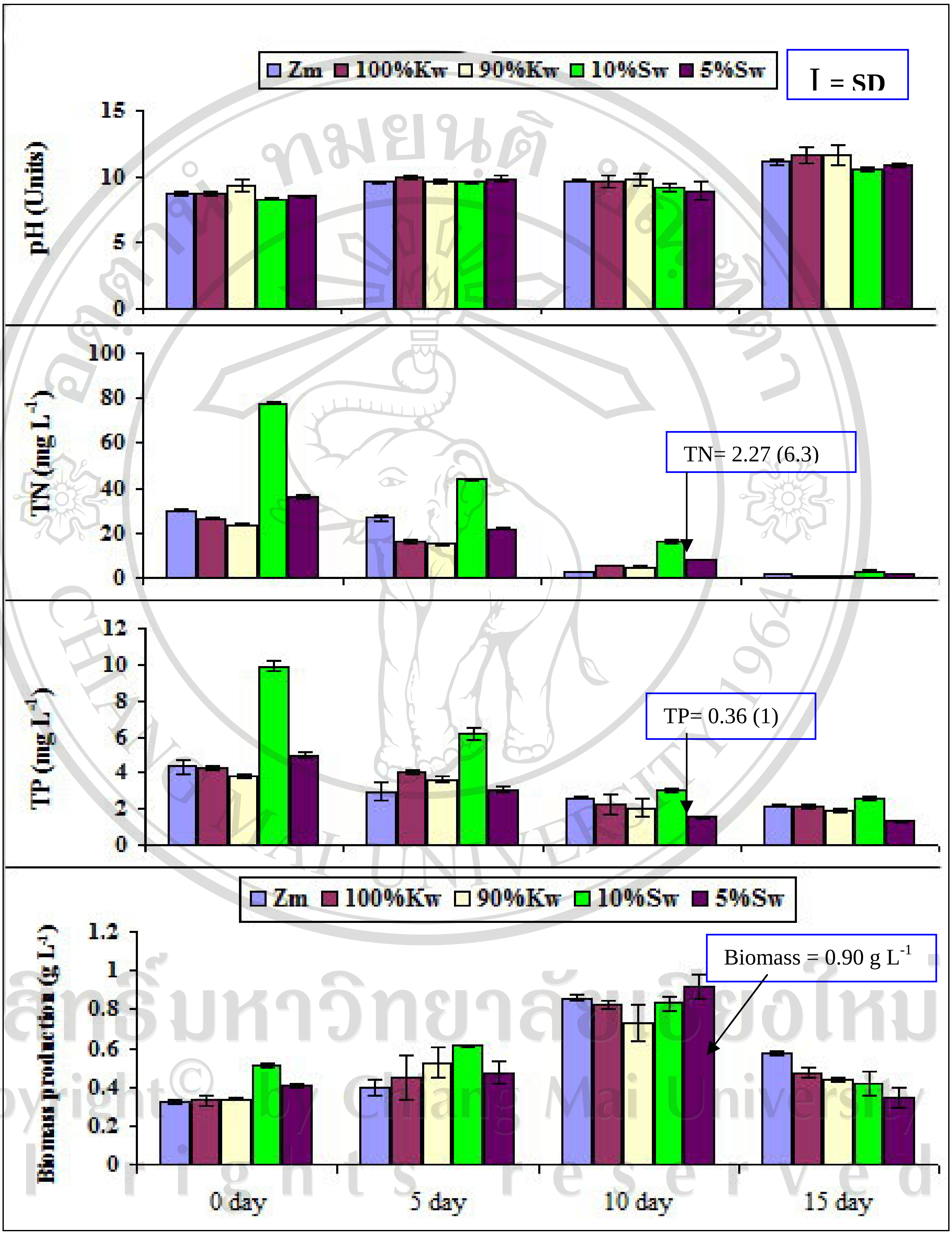

Figure 23 Changes of pH, TN, TP and dry weight biomass of *S. platensis* cultured in modified Zm, Kw and Sw media in the outdoor experimental wastewater for 15 days

4.1.2.2 The pigment contents, γ- linoleic acid, production variable cost, dry weight and nutritional values of *S. platensis*

β-carotene, C-phycocyanin and γ- linoleic acid

It was found that β-carotene and C-phycocyanin obtained from the culture of *S. platensis* in 10%Sw medium was significantly higher than that in modified Zm, 100%Kw, 90%Kw and 5%Sw media with $p< 0.05$. The γ- linoleic acid obtained from *S. platensis* cultured in modified Zm medium was also significantly higher than that in 100%Kw, 90%Kw, 10%Sw and 5%Sw media with $p< 0.05$ (Appendix B. Table 10). The comparison of those values among different media is shown in Figure 24.

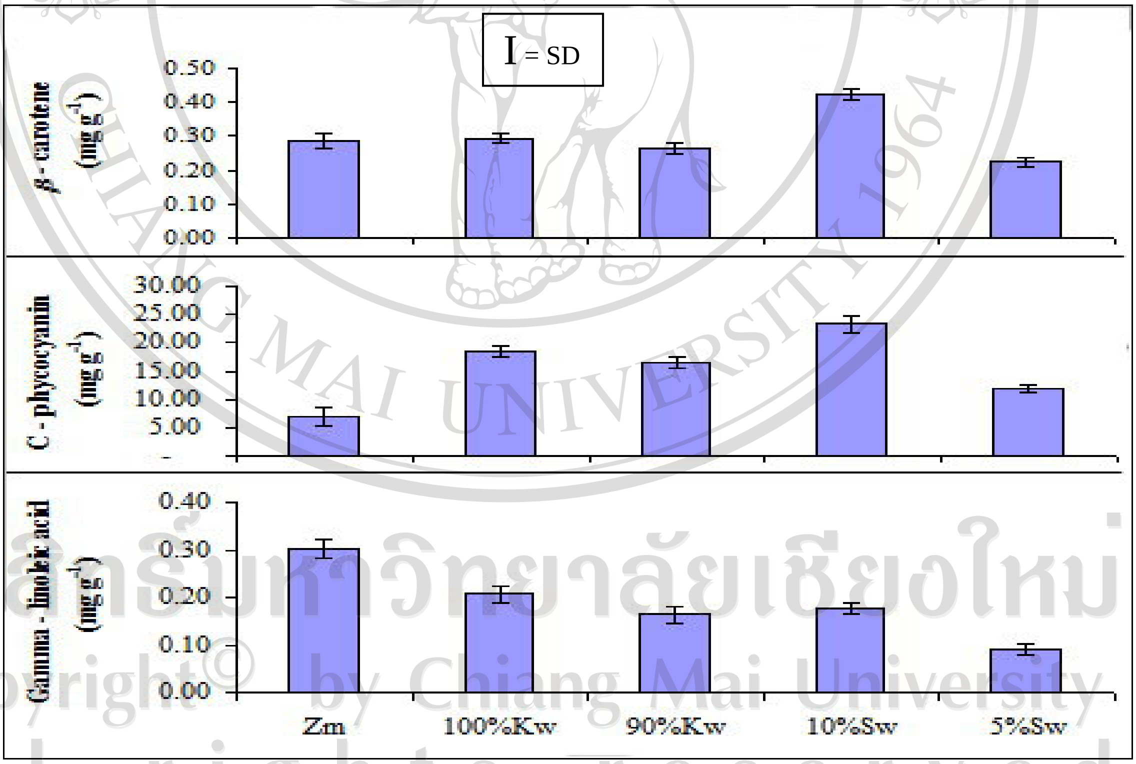

Figure 24 The analysis of β-carotene, C-phycocyanin and γ- linoleic acid of *S. platensis* cultured in modified Zm, 100%Kw, 90%Kw, 10%Sw and 5%Sw media for 15 days

Biomass production and production variable costs of *S. platensis*

The biomass production (dry weight) and production variable costs for various culturing media of *S. platensis* were estimated (De Silva *et al*, 1986) and are summarized in (Appendix B. Table 11) and Figure 25.

Nutritional values

Nutritional values listed in (Appendix B. Table 10) varied considerably among the five used media and comparison is included in the following discussion. Comparison of the values obtained from different media is also shown in Figure 25.

Summary, of the results obtained from *S. platensis* cultivation in the outdoor concrete experimental tanks indicated that the highest algal biomass production was also obtained at day 10 of the cultures in 5%Sw (0.90 g L^{-1} and N : P = 6.3 : 1) which was significantly higher than the cultures in 10%Sw, modified Zm, 100%Kw and 90%Kw, respectively ($p<0.05$). In more detailed results, the highest pigment of β-carotene and C-phycocyanin in the cultures with 10%Sw (0.42 mg g^{-1} and 23.32 mg g^{-1}), while the highest γ- linoleic acid production was found in the cultures with modified Zm (0.302 mg g^{-1}). The production cost of *S. platensis* cultivation in the cultures with modified Zm was 310.60 bath kg^{-1} dried weight.

For the productions of other nutritional parameters of *S. platensis* cultivation, it was found that the highest protein produced in the cultures with modified Zm (54.44%) with moisture content of 10.95%. However, the highest carbohydrate or nitrogen free extract (NFE) production was found in *S. platensis* cultured in 5%Sw (51.38%), while the highest fat, fiber and ash were produced in the cultures with 100%Kw (3.13, 10.65 and 27.94%).

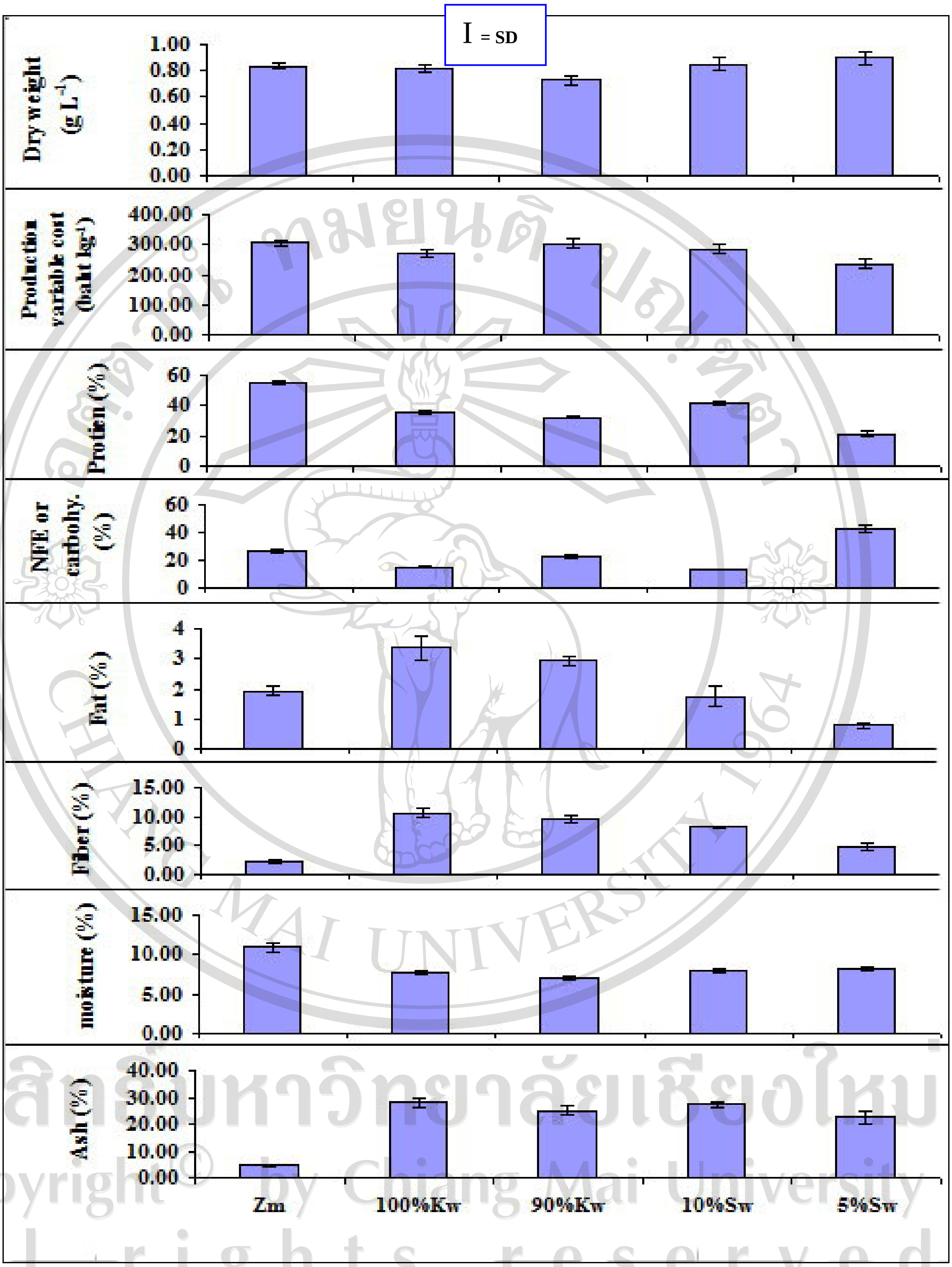

Figure 25 The analysis of dry weight, production variable costs and nutritional values of *S. platensis* cultured in modified Zm, 100%Kw, 90%Kw, 10%Sw and 5%Sw media for 15 days

4.1.2.3 Phytoremediation of wastewater by *S. platensis*.

Water quality parameters for modified Zm, Kw and Sw media used as culturing media filtrate for *S. platensis* are summarized in (Appendix B. Table 12 and 13) and the progress of the changes is illustrated in Figure 26 and 27.

After 15-day experimental period (evaporation of water level 3 cm/ week), several water quality parameters had changed significantly. The figures indicated pH values in modified Zm, 100%Kw, 90 %Kw, 10%Sw and 5%Sw media had increased from 8.73, 9.30, 9.54, 8.33, and 8.51 to 9.73, 9.71, 9.77, 9.12 and 8.92, respectively. Dissolved oxygen (DO) in modified Zm, 100%Kw, 90%Kw, 10%Sw and 5%Sw media had also increased from 3.73, 0.20, 0.30, 0.15 and 0.21 mg L^{-1} to7.20, 6.12, 7.0, 6.09 and 6.60 mg L^{-1}, respectively. In addition, biochemical oxygen demand (BOD) in modified Zm, 100%Kw, 90%Kw, 10%Sw and 5%Sw media had decreased from 9.99, 18.71, 16.84, 169.33 and 126.47 mg L^{-1} to 6.11, 5.15, 4.87, 9.33 and 4.70 mg L^{-1}, respectively (Figure 26 and Appendix B. Table 12, 13).

Chemical oxygen demand (COD) in modified Zm, 100%Kw, 90%Kw, 10%Sw and 5%Sw media had also decreased from 13.21, 28.07, 25.26, 254.43 and 186.50 mg L^{-1} to 7.20, 7.69, 6.93, 14.94 and 7.44 mg L^{-1}, respectively, while the TP values in all 5 media had also decreased from 4.35, 4.25, 3.83, 9.92 and 4.96 mg L^{-1} to 2.17, 2.1, 1.90, 2.58 and 1.29 mg L^{-1}, respectively. The NO_3-N in modified Zm, 100%Kw, 90%Kw, 10%Sw and 5%Sw media had decreased from 30.73, 3.80, 3.47, 31.43 and 11.47 mg L^{-1} to 1.78, to 0.03, 0.027, 0.07 and 0.03 mg L^{-1}, respectively. The NO_2-N in modified Zm, 100%Kw, 90%Kw, 10%Sw media and 5%Sw media had decreased from 0.003, 0.009, 0.008, 0.07 and 0.05 mg L^{-1} to 0.001, 0.003, 0.002, 0.01 and 0.01 mg L^{-1}, respectively. The NH_3-N in modified Zm, 100%Kw, 90%Kw, 10%Sw and 5%Sw media had decreased from 0.03, 7.03, 5.75, 12.08 and 9.37 mg L^{-1} to 0.01, 0.14, 0.13, 0.28 and 0.14 mg L^{-1}, respectively, with most of the changes occurred between day 5, day 10 and day 15 of the cultivation. Organic N (ON) in modified Zm, 100%Kw, 90%Kw, 10%Sw and 5% Sw media had decreased from 0.04, 15.53, 14.55, 33.38 and 14.97 mg L^{-1} to 0.02, 0.54, 0.48, 2.68 and 1.34 mg L^{-1}, respectively. Total oxidized N (TON) in modified Zm, 100%Kw, 90%Kw, 10%Sw and 5%Sw media had decreased from 30.73, 3.81, 3.48, 31.50 and 11.52 mg L^{-1} to 1.78, 0.033, 0.029, 0.08 and 0.04 mg L^{-1}, respectively. TKN values in modified Zm, 100%Kw, 90%Kw, 10%Sw and 5%Sw media had decreased from 0.07, 22.56, 20.3, 45.46 and 24.34 mg L^{-1} to 0.05, 0.68, 0.61, 2.96 and 1.48 mg L^{-1}, respectively. Total-N (TN) in modified Zm, 100% Kw, 90% Kw, 10% Sw and 5% Sw media had decreased from 30.80, 26.37, 23.78, 77.68 and 35.86 mg L^{-1} to 1.83, 0.71, 0.64, 3.04 and 1.52 mg L^{-1}, respectively (Appendix B. Table 12 and 13) with most of the changes occurred between day 10 and day 15 of the cultivation (Figure 27).

At the end of the experiments, using 100%Kw, 90%Kw, 10%Sw and 5%Sw media, values decreased as; biological oxygen demand (BOD) for 72.74%, 71.08%, 94.50% and 96.28%; chemical oxygen demand (COD) for 72.6%, 73%, 94% and 96%; NH_3-N for 98%, 97.8%, 97.66% and 98.5%; ON for 96.52%, 96.7%, 91.97% and 91.05%; TKN for 97%, 97%, 97% and 97%; NO_3-N for 99.2%, 99.2%, 99.77% and 99.2%; TON for 99.15%, 99.2%, 99.75% and 99.65%; and TN for 97.3%, 97.3%, 96% and 96%, respectively. BOD, COD, nitrogen and phosphorus levels in 100%Kw, 90%Kw, 10%Sw and 5%Sw media are significantly decreased compared to those values in modified Zm medium with $p<0.05$ (Appendix B. Table 12 and 13).

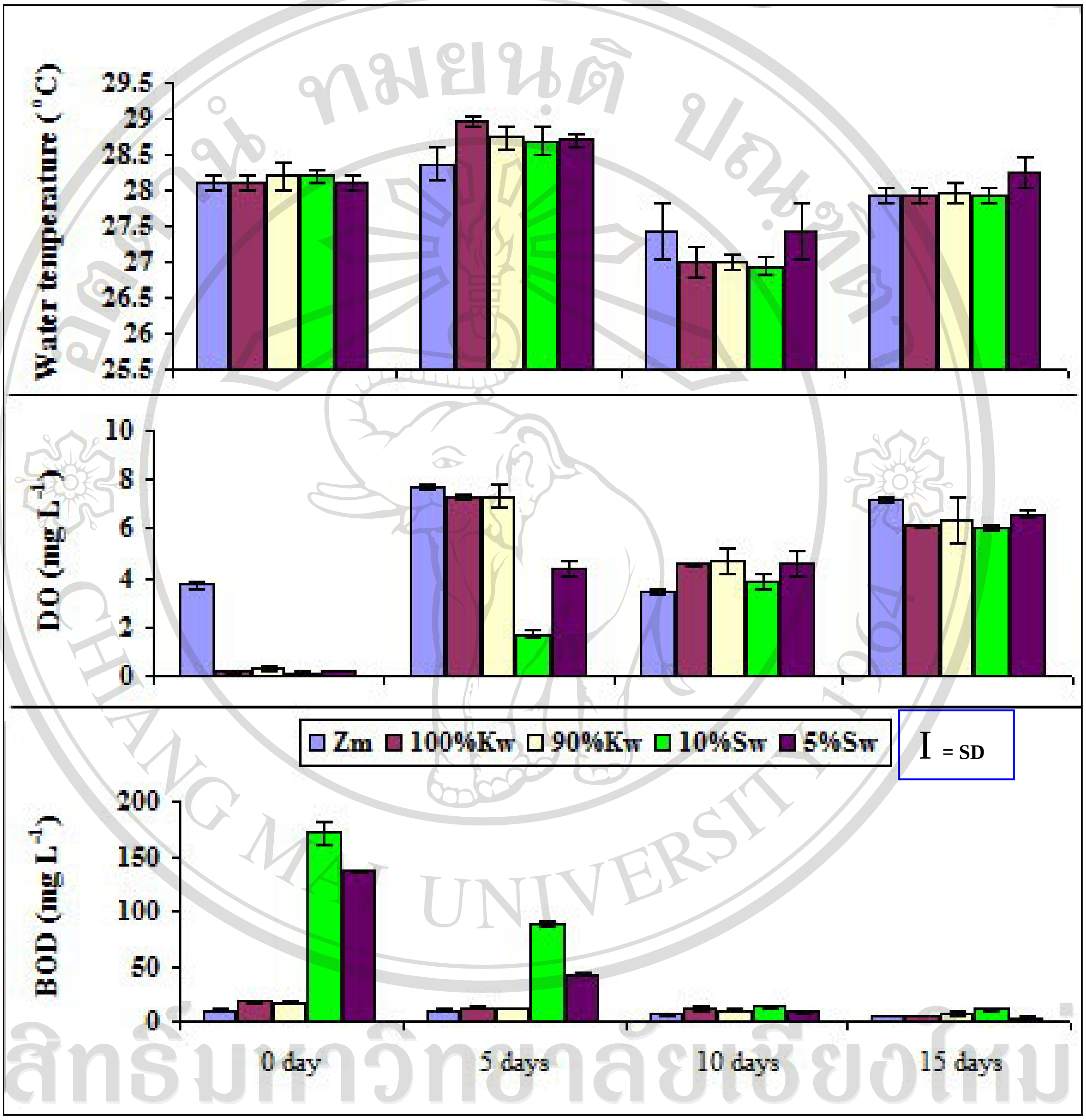

Figure 26 Changes of water temperature, DO and BOD of *S. platenssis* cultured in wastewater for 15 days

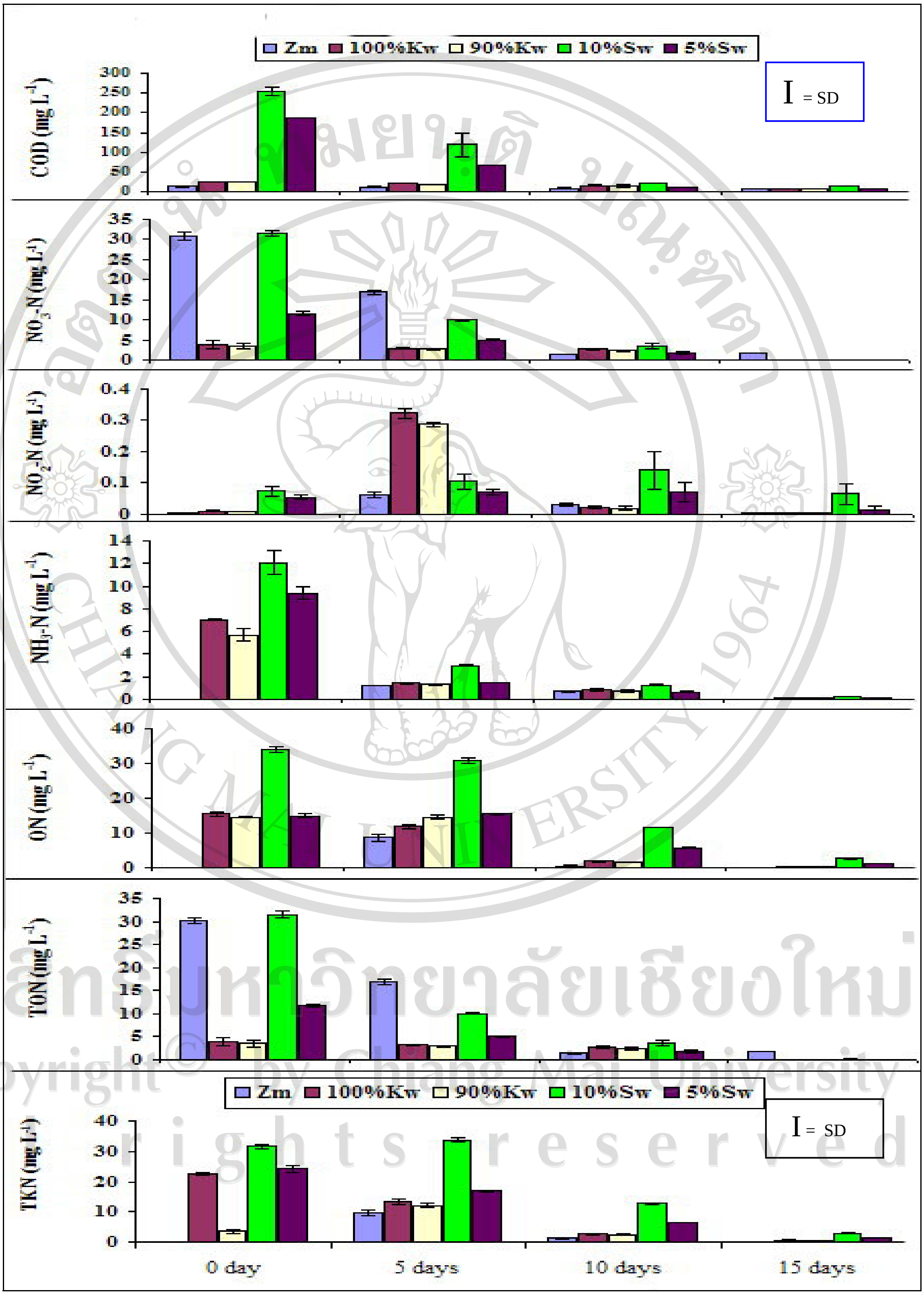

Figure 27 Changes of COD, NO_3-N, NO_2-N, NH_3-N, ON, TON and TKN of *S. platensis* cultured in wastewater for 15 days

4.1.3 Mass cultures of *S. platensis* in cement raceway ponds and earthen raceway ponds

Oil-extracted soybean fermented water (10%Sw) was the medium selected from the outdoor mass culture experiments, for the reason that it gave the most preferable results in all parameters analysed. The medium was firstly diluted with tap water (Sw : tap water = 1 : 9 made up 10%Sw), then 0.1 g L^{-1} of $NaHCO_3$ was added. After that, *S. platensis* culture took place in cement raceway ponds and earthen raceway ponds where the biomass production, water quality and nutritional values were measured and compared among the experiment units.

4.1.3.1 Water quality in cement raceway ponds and earthen raceway ponds

As the results, values of water quality parameters in the experimental cement raceway ponds and earthen raceway ponds are as follows. The air temperature 28 to 33^{o}C and water temperature ranged from 26.8 to 30 ^{o}C, whereas pH values ranged between 8.33 and 9.90, and DO ranged from 0.14 to 3.93 mg L^{-1}. BOD varied from 19.27 to 169.33 mg L^{-1}, while COD were between 29.18 and 254.40 mg L^{-1}, NO_3-N were between 5.17 and 31.38 mg L^{-1}, while NO_2-N ranged from 0.03 to 0.09 mg L^{-1}, and NH_3-N ranged between 1.25 and 11.25 mg L^{-1}, TP varied from 2.90 to 9.46 mg L^{-1} and optical density (OD) varied from 0.41 to 0.74 units (Figure 28). Water quality of *S. platensis* cultures were not-significantly different between cement raceway ponds and earthen raceway ponds after 10-day cultivation (Appendix C, Table 44 - 46).

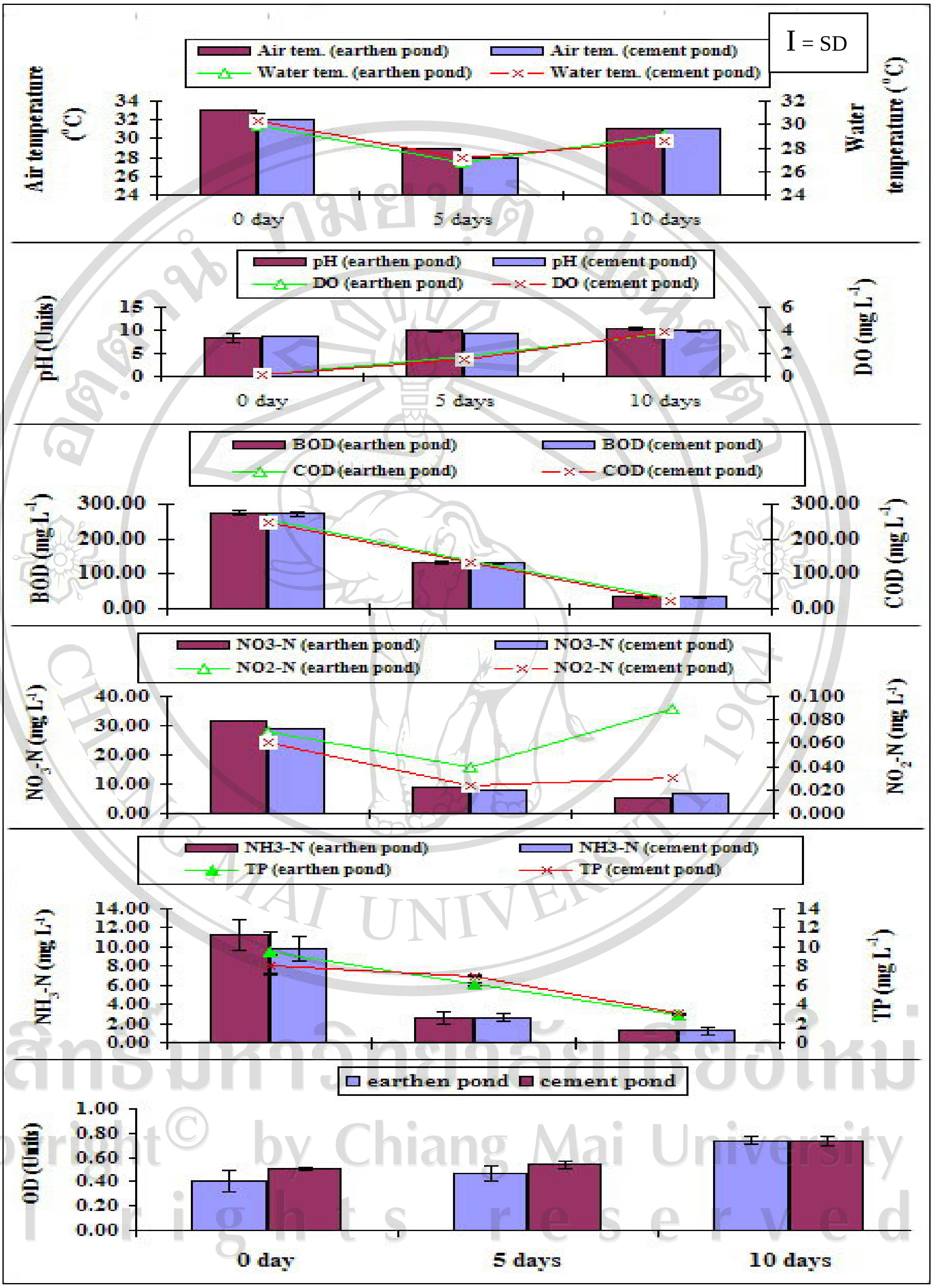

Figure 28 Changes in air and water temperature, pH, DO, BOD, COD, NO_3-N, NO_2-N, NH_3-N, TP and optical density (OD) of *S. platensis* cultured in the wastewater for 10 days

4.1.3.2 Biomass production (dry weight), production variable costs and nutritional values of *S. platensis* in raceway ponds

Biomass production (dry weight) and production variable cost of *S. platensis* in various culturing media were estimated (De Silva *et al.*, 1986) and compared between cement raceway ponds and earthen raceway ponds. The results were summarized as shown in Table 4. Nutritional values listed in Table 4 varied considerably among the second media and comparisons are included in the following discussion.

In the comparison between the cultivations of *S. platensis* cultured in cement raceway ponds and earthen raceway ponds, the biomass production (range of 0.80-0.85 g L^{-1}), production cost (range of 221.58-240.00 baht kg^{-1}), and protein production (range of 39.40-40.82%) were not statistically different (Table 4).

Table 4. Statistical summary (mean ± SD) for the analysis of biomass production (dry weight), production variable cost and nutritional values of *S. platensis* cultured in 10%Sw medium for 10 days

Parameter	**cement raceway pond 10%Sw**	**earthen raceway pond 10%Sw**
Biomass production (dry weight ; g L^{-1})	0.85 ± 0.07^{ns}	0.80 ± 0.05^{ns}
production variable cost (baht kg^{-1})	221.58 ± 13.13^{ns}	240.68 ± 15.47^{ns}
Protein (%)	40.82 ± 2.19^{ns}	39.40 ± 1.50^{ns}
Carbohydrate (%)	13.82 ± 0.92^{ns}	12.46 ± 0.15^{ns}
Fat (%)	1.67 ± 0.19^{ns}	1.60 ± 0.08^{ns}
Fiber (%)	8.40 ± 0.68^{ns}	9.04 ± 0.02^{ns}
Moisture (%)	8.17 ± 0.24^{ns}	8.01 ± 0.27^{ns}
Ash (%)	27.12 ± 1.15^{ns}	29.49 ± 1.09^{ns}

4.2 Tuptim tilapia culture

4.2.1 Nursing larval Tuptim tilapia in the earthen pond using raw *S. platensis*

4.2.1.1. Growth performance

Average weight (g fish^{-1})

The larval Tuptim tilapia had average initial weight of 0.02± 0.00 g fish^{-1}. At day 0 to 60, the averaged weights of fish in batch control group 5%CD was higher than 1%RS, 3%RS and 5%RS (feed added with raw *S. platensis* to 1%, 3% and 5% as dry weight / body weight/ day). At day 60 to 90, the averaged weights of fish in batch 1%RS, 3%RS and 5%RS were the same to that of the control group CD (commercial diet with 5% CD) (Figure 29 and Appendix B Table 14).

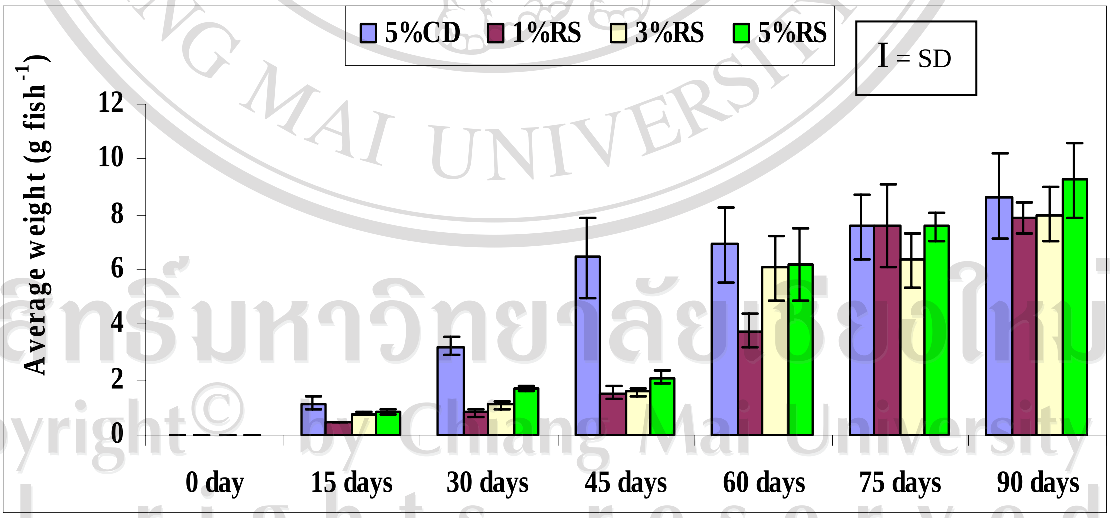

Figure 29 Changes of average weight of larval Tuptim tilapia fed with raw *S. platensis* cultured in the earthen pond for 90 days

Weight gain percentage (%) and specific growth rate (%/day)

Weight gain percentage and specific growth rate from nursing larval Tuptim tilapia with all experimental treatments were non-significantly different at 90 days (Figure 30 and Appendix B Table 15).

Survival rate (%)

Survival rate of nursing larval Tuptim tilapia with 5%CD and 5%RS were significantly higher than 3%RS and 1%RS with $p<0.05$ for 90 days (Figure 30 and Appendix B Table 15).

Feed conversion ratio (units) and protein efficiency ratio (units)

The lowest feed conversion ratio of nursing larval Tuptim tilapia were achieved in 3%RS after 90 days. The highest protein efficiency ratio of nursing larval Tuptim tilapia were achieved in 5%CD, 5%RS and 3%RS after 90 days (Figure 30 and Appendix B. Table 15).

Although, nursing of larval Tuptim tilapia in the earthen pond, using raw *S. platensis* form different diet-treatments including 5% commercial diets (5%CD) as control, 1% raw *S. platensis* (1%RS), 3% raw *S. platensis* (3%RS) and 5% raw *S. platensis* (5%RS), the present study resulted as 5%CD had enhanced the highest average weight, weight gain percentage and specific growth rate in the larval fish during the first 60 days of the experiment (day-0 to day-60), however, during day-60 and the completion at day-90, the resulted performances of all the feeding formulas were not statistically different. This indicated that the raw *S. platensis* was a promising partial in feeding formula for nursing larval Tuptim tilapia.

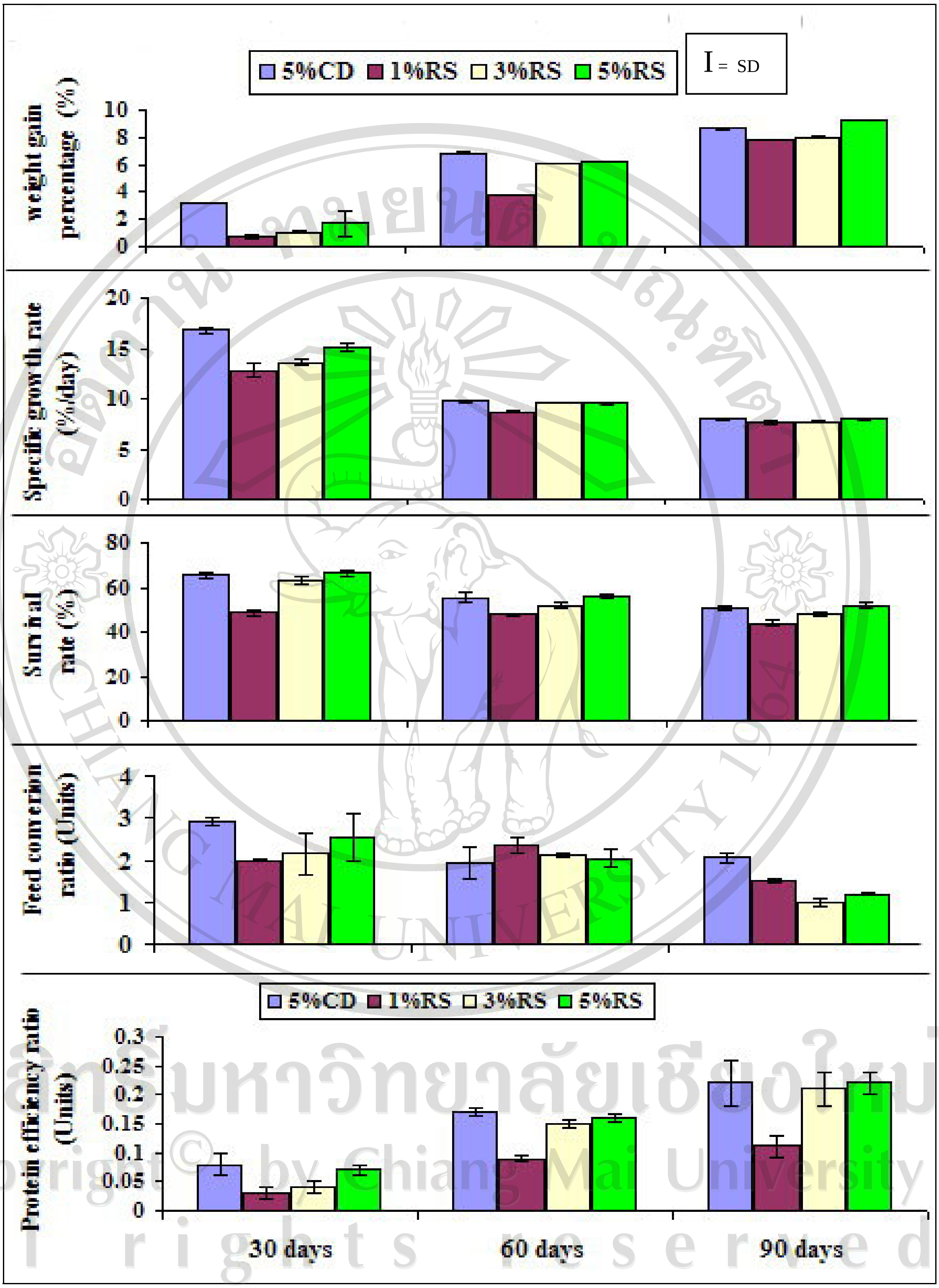

Figure 30 The analysis of weight gain percentage (%), specific growth rate (% /day), survival rate (%), feed conversion ratio (units) and protein efficiency ratio (units) of larval Tuptim tilapia nursing for 90 days

4.2.1.2 The estimation of larval Tuptim tilapia immunity with the treatment of using raw *S. platensis* as feed in the earthen pond

The immunity, measured by decreasing unit of turbidity 0.001 unit/minute, which means one unit of lysozyme activity, of nursed larval Tuptim tilapia with 3%RS feeding formula was significantly lower than 5%CD, 5%RS and 1%RS with $p < 0.05$ after nursing for 60 days. The best immunity as unit of lysozyme activity of nursing larval Tuptim tilapia was achieved in 3%RS and 5%RS feeding formulas after 90 days nursing. Red blood cell and white blood cell count of nursed larval Tuptim tilapia with 5%RS was significantly higher than fish from 3%RS, 1%RS and 5%CD (control group), with $p < 0.05$ after 90 days nursing (Figure 31 and Appendix B. Table 16).

In addition, the result was also clear that the highest immunity of nursed larval tuptim tilapia, measured as unit of lysozyme activity, red blood cell and white blood cell counts, was determined in the larval fish fed with 5%RS.

4.2.1.3 Analyses for physico-chemical qualities of water where larval Tuptim tilapia were nursed in the earthen pond (Table 14)

The results of analyses for physico-chemical qualities of larval Tuptim tilapia nursed in the earthen pond with 4 different feeding formulas, 5%CD, 1%RS, 3%RS and 5%RS for 90 days are as follows.

Air temperature and water temperature: With all four experimental treatments including control group (5%CD), the water temperature and air temperature of the water in the ponds nursing larva Tuptim tilapia were non-significant different (Appendix B. Table 17).

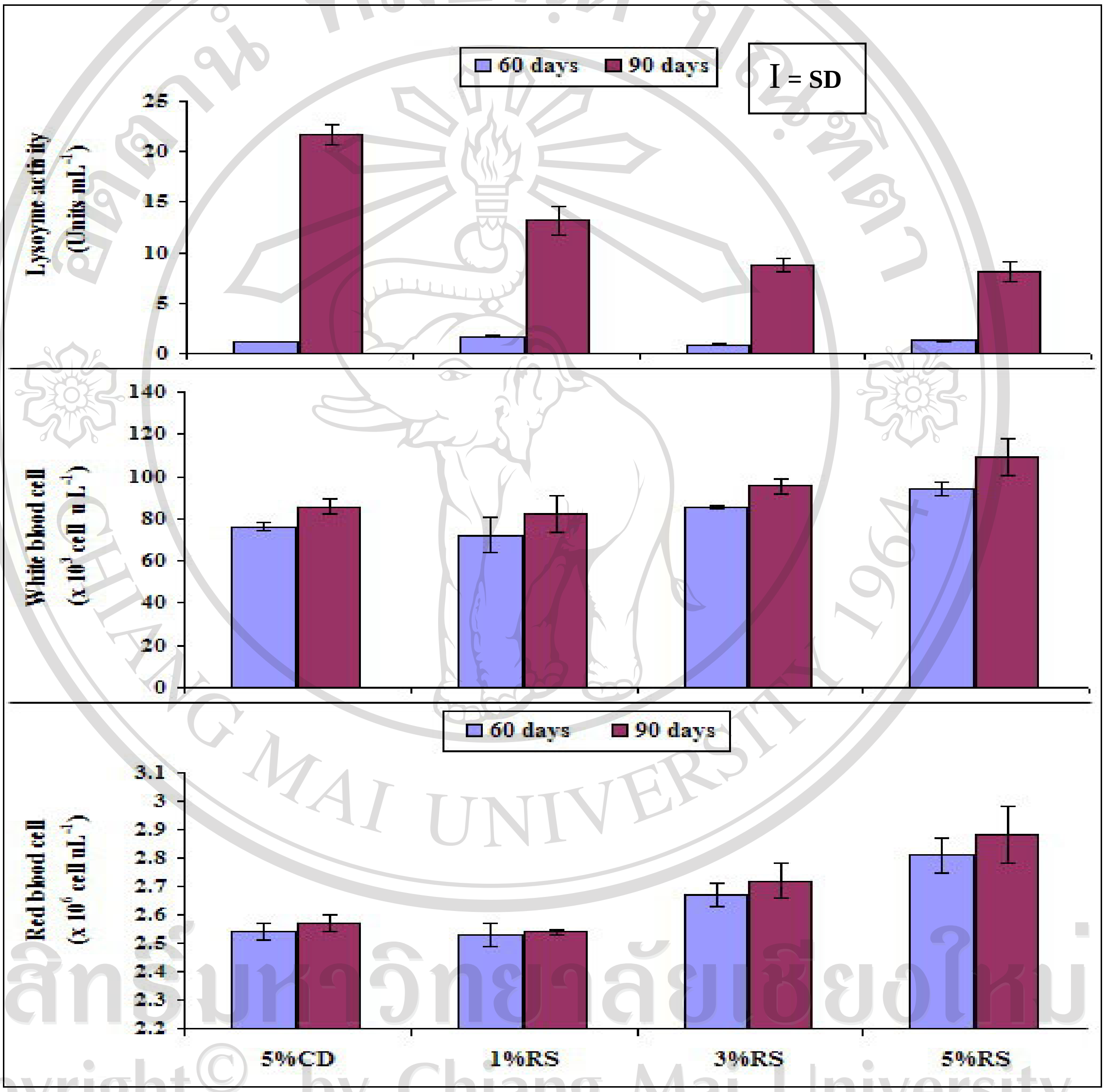

Figure 31 The analysis of lysozyme activity as turbidity (units mL^{-1}), red blood cell ($x10^{6}$ cell uL^{-1}), white blood cell ($x10^{3}$ cell μL^{-1}) count of larval Tuptim tilapia nursing for 90 days

pH: The highest pH in larval Tuptim tilapia ponds were achieved from 5%RS and 3%RS batches after 90 days nursing (Figure 32 and Appendix B. Table 17).

Alkalinity (mg L^{-1}): The alkalinity in the larval Tuptim tilapia nursing ponds with 5%CD was significantly higher than those of 1%RS, 5%RS and 3%RS with $p< 0.05$ after 30 days nursing. The highest alkalinity in larval Tuptim tilapia nursing ponds were achieved from 5%RS, 3%RS and 1%RS after 60 and 90 days nursing (Figure 32 and Appendix B. Table 17).

Total dissolved solid (TDS; mg L^{-1}): Throughout all four experimental treatments, TDS in larval Tuptim tilapia nursing ponds were measured non-significant different after 30 days nursing. The TDS in larval Tuptim tilapia nursing ponds with 3% RS and 5% RS were significantly higher than that of 1% RS and 5% CD with $p< 0.05$ after 60 days. The highest TDS in larval Tuptim tilapia nursing pond was achieved from 5% RS after 90 days nursing (Figure 32 and Appendix B Table 17).

Conductivity (μS cm^{-1}): Throughout all four treatments, the conductivity in larval Tuptim tilapia nursing ponds were non-significant different after nursing for 30 days. The conductivity in larval Tuptim tilapia nursing pond with 5%RS and 3%RS were significantly higher than that in 1%RS and 5%CD with $p< 0.05$ after 60 and 90 days nursing (Figure 32 and Appendix B. Table 17).

Dissolved Oxygen (DO; mg L^{-1}): Throughout all four treatments, DO values in larval Tuptim tilapia nursing ponds were non-significant different at 30 and 60 days. The DO values in the larval Tuptim tilapia nursing pond with 5%CD was significantly higher than those of 1% RS, 3% RS and 5% RS with $p< 0.05$ at 90 days (Figure 32 and Appendix B. Table 17).

Orthophosphates - phosphorus (PO_4-P; mg L^{-1}): Throughout all four treatments, PO_4-P in larval Tuptim tilapia nursing ponds were non-significant different at 30 days. The PO_4-P value in larval Tuptim tilapia nursing pond with 5%RS was significantly higher than those of 3%RS, 1%RS and 5%CD with $p< 0.05$ at 60 days. The highest PO_4-P values in larval Tuptim tilapia nursing pond were achieved from 5%RS, 3%RS and 1%RS after nursing for 90 days (Figure 32 and Appendix B. Table 17).

Ammonia - nitrogen (NH_3-N; mg L^{-1}): The NH_3-N in larval Tuptim tilapia nursing pond with 5%RS was significantly higher than those of 3% RS, 1% RS and 5% CD with $p< 0.05$ after nursing for 90 days (Figure 32 and Appendix B. Table 17).

Summary, of the physico-chemical parameters as quality of water used for nursing larval Tuptim tilapia in earthen ponds, it indicated the air temperature of 30.00 - 31.00 ^{o}C, water temperature of 27.03 - 31.33 ^{o}C, water pH of 7.17 - 9.07, alkalinity of 70.87 - 112.75 mg g^{-1}, TDS of 113.33-140.00 mg g^{-1}, conductivity of 213.33 - 265.33 μS cm^{-1}, DO of 5.70 - 8.13 mg g^{-1}, PO_4-P of 0.003 - 0.005 mg g^{-1}, NH_3-N of 0.010 - 0.017 mg g^{-1}, which revealed the water quality of the nursing ponds was with in the standard values (Tave, 1990).

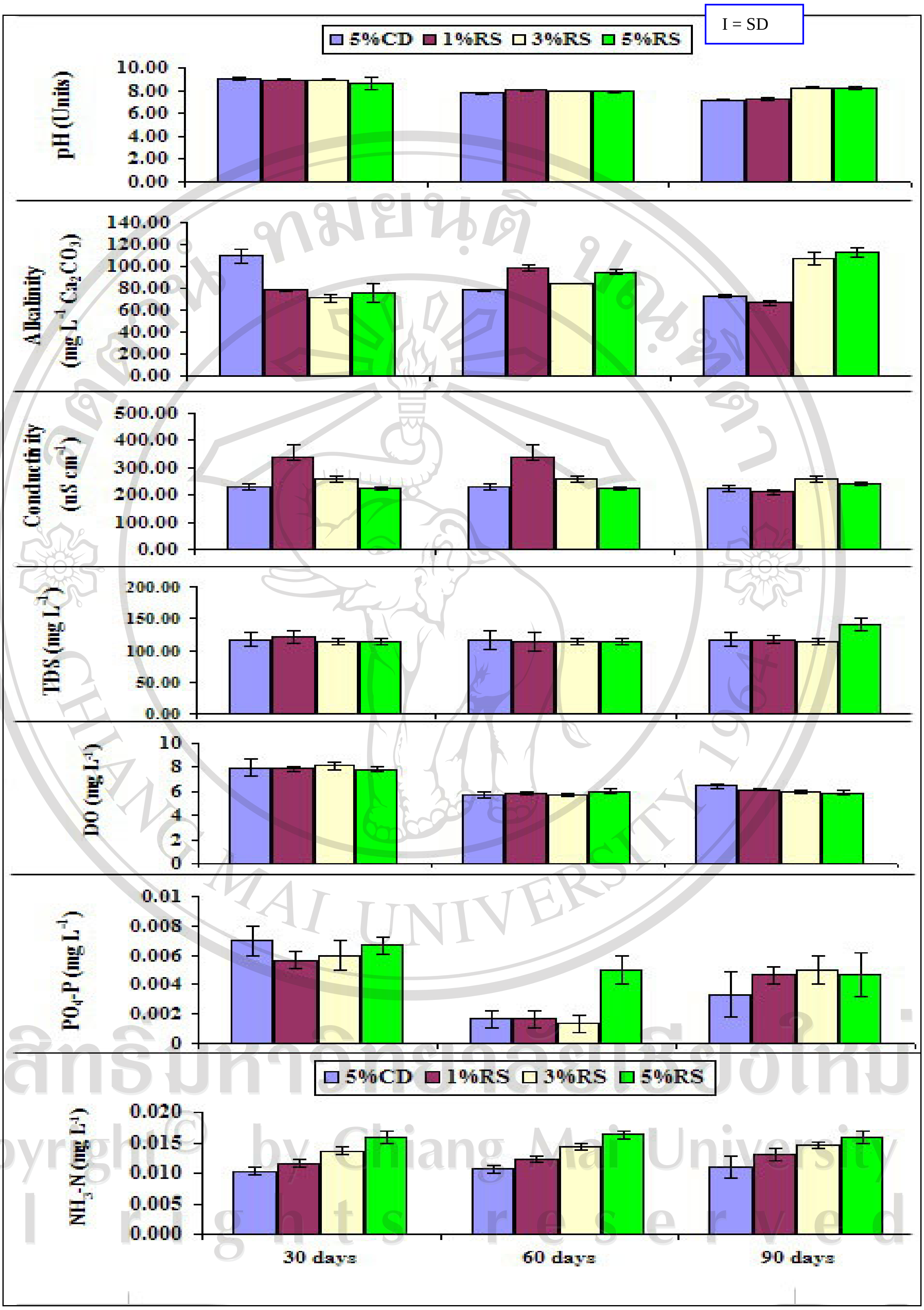

Figure 32 The analysis of pH, alkalinity, conductivity, TDS, DO, PO_4-P and NH_3-N using raw *S. platensis* as feeding formulas in larval Tuptim tilapia nursing pond for 90 days

4.2.2 Culturing male and female juvenile Tuptim tilapia in the earthen pond

4.2.2.1 Growth performance of male juvenile Tuptim tilapia

Average weight (g fish^{-1})

Male juvenile Tuptim tilapia had initial average weight ranged from 21.00 ± 1.00 to 27.67 ± 2.08 g fish^{-1}. The highest average weight of the cultured male juvenile Tuptim tilapia was achieved in 55%RS the 60 and 90–day. The average weight of the cultured male juvenile Tuptim tilapia with all experimental treatments were non-significantly different the 120 and 150–day (Figure 33 and Appendix B. Table18).

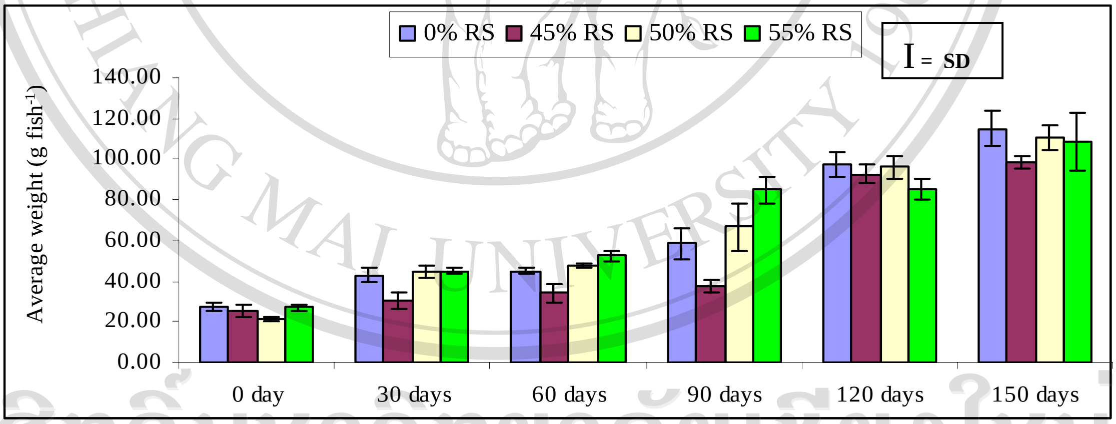

Figure 33 Changes of average weight of male juvenile Tuptim tilapia using raw *S. platensis* feed culture in the earthen pond for 150 day.

Weight gain percentage (%)

Weight gain percentage from the cultured male juvenile Tuptim tilapia with all experimental treatments were non-significantly different (Figure 34 and Appendix B. Table19).

Specific growth rate (% / day)

The highest specific growth rate of the cultured male juvenile Tuptim tilapia was achieved in 50%RS after 150 days (Figure 34 and Appendix B. Table19).

Survival rate (%)

Survival percentage of the cultured male juvenile Tuptim tilapia within all experimental treatments were non-significantly different (Figure 34 and Appendix B. Table19).

Feed conversion ratio (units)

The lowest feed conversion ratio of the cultured male juvenile Tuptim tilapia were achieved in 45%RS and the control group (0%RS) after 150 days (Figure 34 and Appendix B. Table19).

Protein efficiency ratio (units)

Protein efficiency ratio of the cultured male juvenile Tuptim tilapia with 55%RS and 50%RS were significantly higher than 45%RS and the control group (0%RS) with $p < 0.05$ at days 150 (Figure 34 and Appendix B. Table19).

The result of culturing male juvenile Tuptim tilapia with four different diet-treatments, including commercial diets with 0%RS, 45%RS, 50%RS and 55%RS indicated no different, where the experimented male juvenile Tuptim tilapia fed with 55%RS and 50%RS had shown the highest protein efficiency ratio.

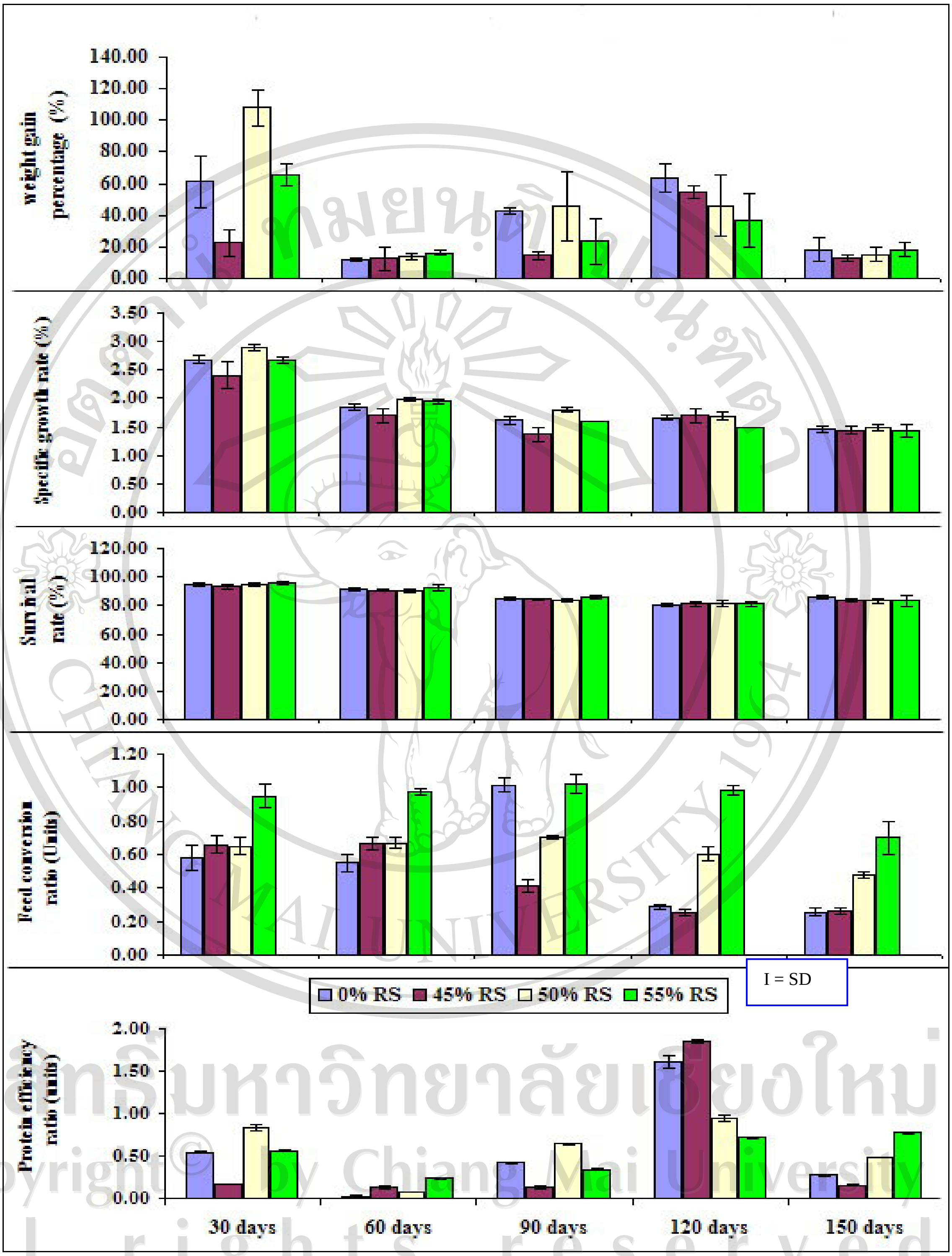

Figure 34 The analysis of weight gain percentage (%), specific growth rate (%), survival rate (%), feed conversion ratio (units), protein efficiency ratio (units) of the cultured male juvenile Tuptim tilapia being raised for 150 day.

4.2.2.2 Growth performance of female juvenile Tuptim tilapia

Average weight (g fish^{-1})

Female juvenile of Tuptim tilapia had initial weight ranged from 27.67±2.08 to 18.33±1.53 g fish^{-1} while the average weight of fish from 55%RS had showed significant the same figure to that of the control group with 0%RS at day 0–120, but the value from 0%RS (control) was still significantly higher than those of 55%RS, 50%RS and 45%RS with $p<0.05$ after 150 days (Figure 35 and Appendix B. Table 20).

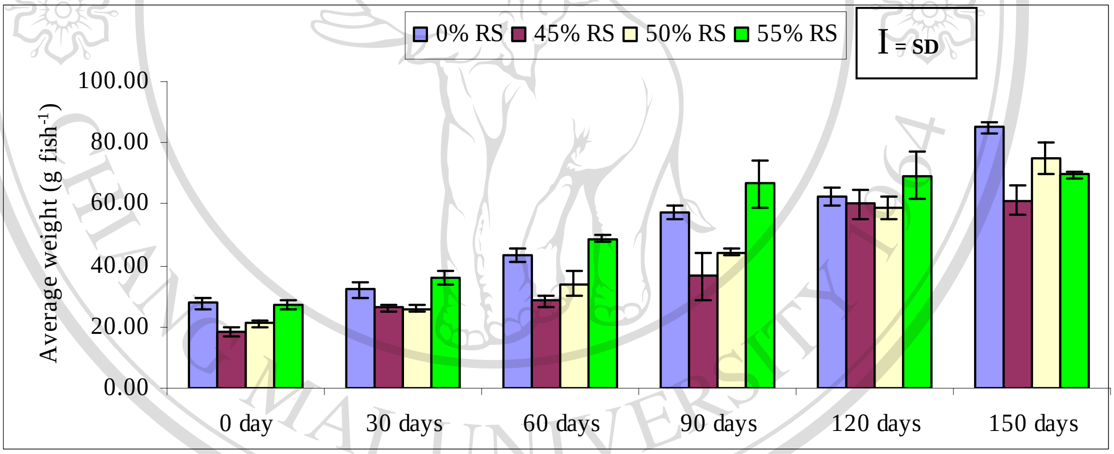

Figure 35 Changes of average weight of female juvenile Tuptim tilapia using raw *S. platensis* feed culture in the earthen pond for 150 days

Weight gain percentage (%)

Weight gain percentage of the cultured female juvenile Tuptim tilapia with 55%RS and 0%RS (control) were significantly higher than those of 50%RS and 45%RS with $p< 0.05$ after being raised for 150 days (Figure 36 and Appendix B. Table 21).

Specific growth rate (% / day)

Specific growth rate of the cultured female juvenile Tuptim tilapia with 55%RS, 0%RS (control) and 50%RS were significantly higher than those of 45%RS with $p< 0.05$ after being raised for 150 days (Figure 36 and Appendix B. Table 21).

Survival rate (%)

Survival rate of the cultured female juvenile Tuptim tilapia with all experimental treatments were non-significantly different (Figure 36 and Appendix B. Table 21).

Feed conversion ratio (units)

The lowest feed conversion ratio of the cultured female juvenile Tuptim tilapia were achieved in 45%RS and 0%RS (control) after being raised for150 days (Figure 36 and Appendix B. Table 21).

Protein efficiency ratio (units)

The highest protein efficiency ratio of the cultured female juvenile Tuptim tilapia were achieved in 55%RS, 5%RS and 0%RS (control) after being raised for 150 days (Figure 36 and Appendix B. Table 21).

The result of culturing female juvenile Tuptim tilapia with the same diet-treatments as male juvenile Tuptim tilapia, including 0%RS, 45%RS, 50%RS and 55%RS, indicated the highest average weight, weight gain percentage and specific growth rate with 0%RS and 55%RS. Whereas the highest protein efficiency ratio in female juvenile Tuptim were found in the fish fed with 0%RS, 55% RS and 50% RS.

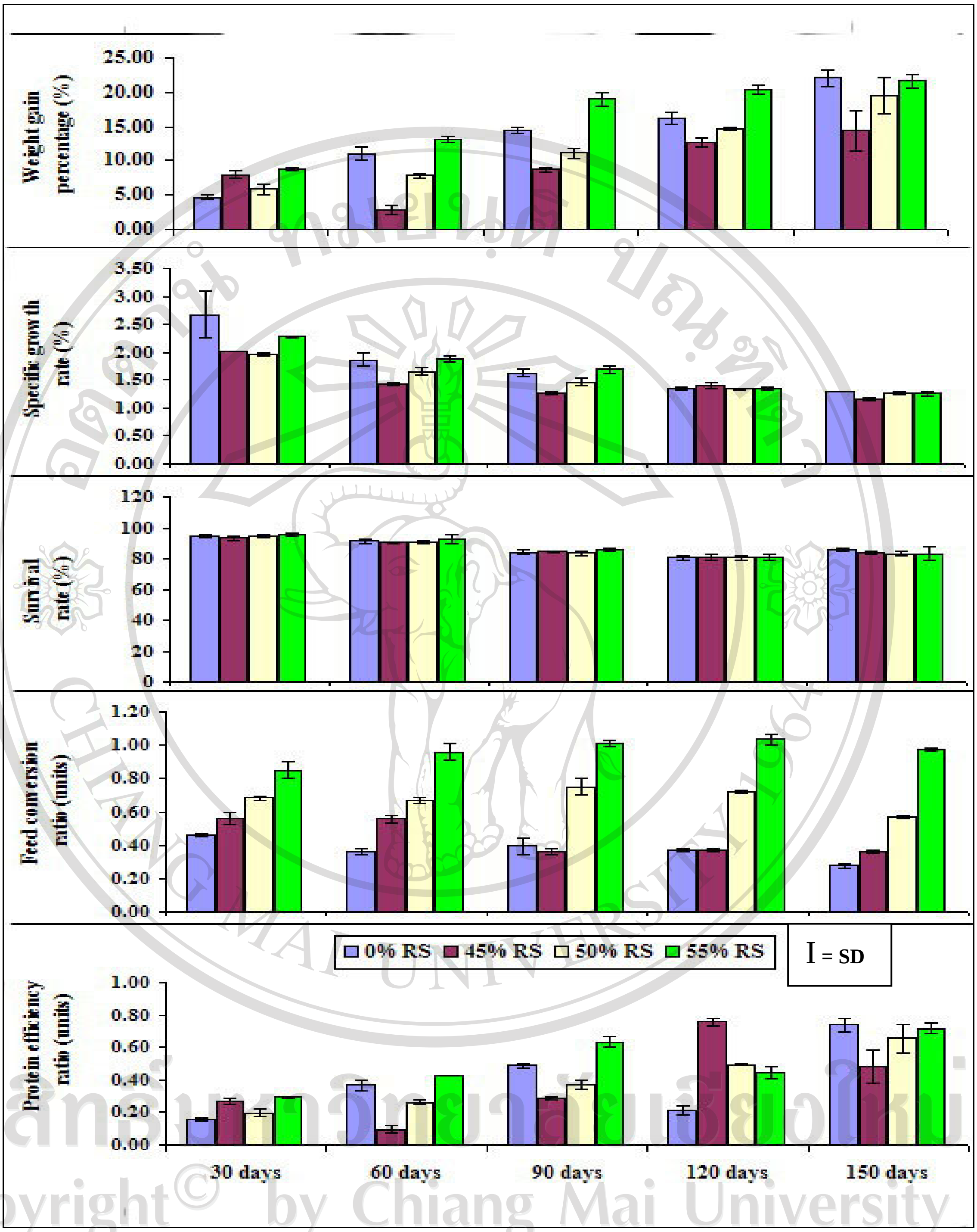

Figure 36 The analysis of weight gain percentage (%), specific growth rate (%), survival rate (%), feed conversion ratio (units), protein efficiency ratio (units) of the cultured female juvenile Tuptim tilapia using raw *S. platensis* feed culturing in the earthen pond for 150 days

4.2.2.3 The estimation of juvenile Tuptim tilapia immunity, using raw *S. platensis* as feeding formulas in the earthen pond

The immunity, as the decreasing unit of turbidity 0.001 unit / minute which means one unit of lysozyme activity, of the cultured juvenile Tuptim tilapia with 50%RS and 55%RS feeding were significantly lower than those of 45%RS and 0%RS (control) with $p< 0.05$ after 30 days. The best immunity as unit of lysozyme activity of the cultured Tuptim tilapia was achieved from 55%RS after 150 days. The red blood cell and white blood cell count from the cultured juvenile Tuptim tilapia with 55%RS was significantly higher than those of 50%RS, 45%RS and 0%RS (control) with $p< 0.05$ after 150 day (Figure 37 and Appendix B. Table 22).

Additionally, the highest immunity of cultured juvenile Tuptim tilapia, measured as unit of lysozyme activity, red blood cell and white blood cell counts, was determined in the fish fed with 55%RS.

4.2.2.4 The pigments, γ- linoleic acid and nutritional values of feed, flesh, eggs and production variable cost of juvenile Tubtim tilapia culture

β-carotene, C-Phycocyanin and γ- linoleic acid

The β-carotene obtained from the feed of juvenile Tuptim tilapia raised in 55%RS was significantly higher than those of 50%RS, 45%RS and 0%RS (control) with $p<0.05$. The β-carotene from the flesh of juvenile Tuptim tilapia cultured with

55%RS and 50%RS were significantly higher than those of 45%RS and 0%RS (control) with $p<0.05$. However, the β-carotene from the eggs of juvenile Tuptim tilapia was non-significantly different after cultured for 150 days (Figure 38 and Appendix B. Table 23).

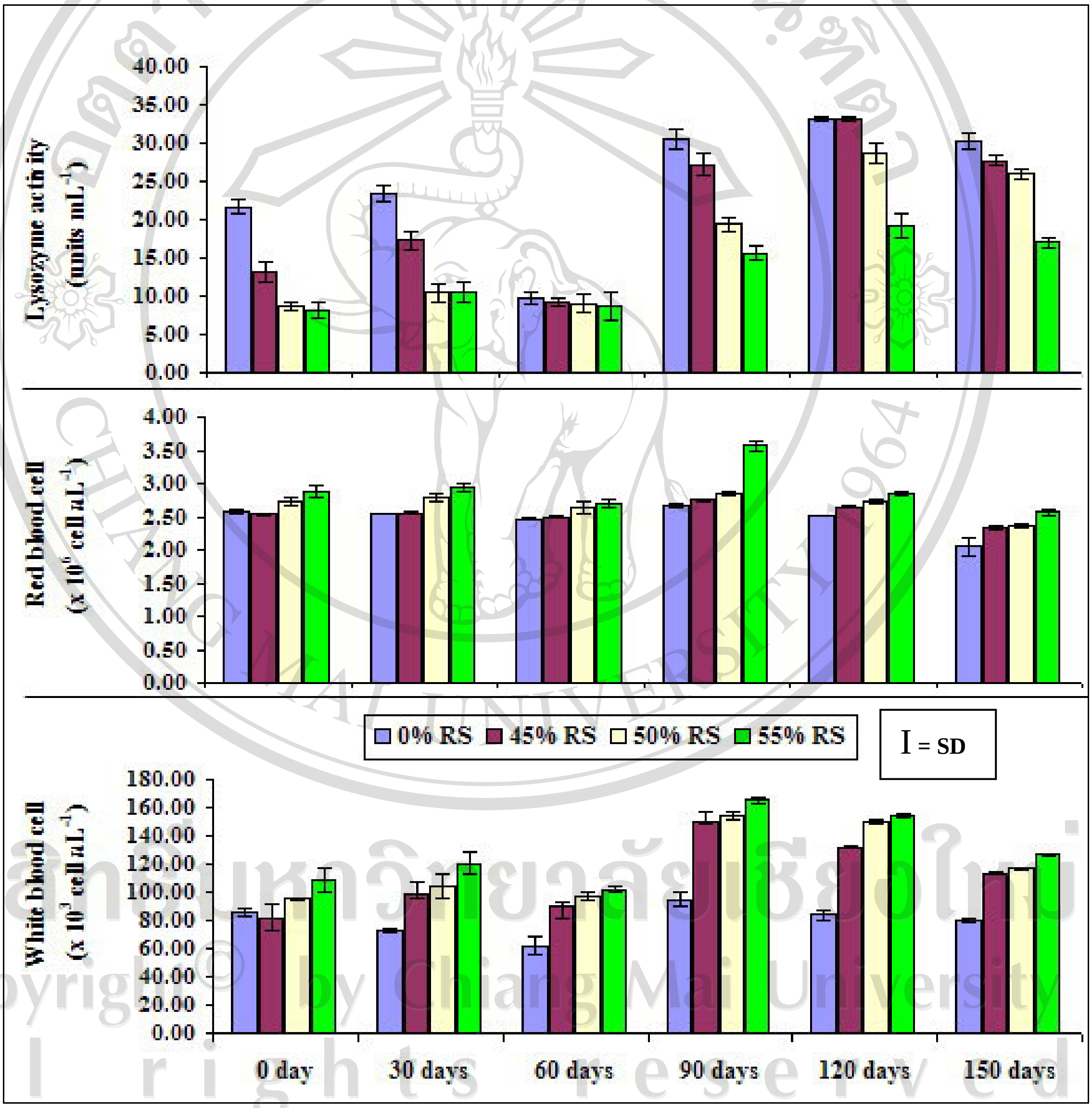

Figure 37 The analysis of lysozyme activity (units mL^{-1}), red blood cell ($x\ 10^6$ cell μL^{-1}) and white blood cell ($x\ 10^3$ cell μL^{-1}) count of juvenile Tuptim tilapia for 150 days

The C-phycocyanin from feed of juvenile Tuptim tilapia cultured with 55%RS was significantly higher than 45%RS, 50%RS and 0%RS. The C-phycocyanin from flesh and eggs of juvenile Tuptim tilapia cultured with 55% RS was significantly higher than those of 50%RS, 45%RS and 0%RS (control) with $p < 0.05$ after cultured for 150 days (Figure 39 and Appendix B. Table 23).

The γ- lnoleic acid from feed and eggs of juvenile tuptim tilapia nursed with 55%RS and 50%RS were significantly higher than those of 45%RS and 0%RS (control) with $p<0.05$, but the γ-linoleic acid from the flesh of juvenile tuptim was non-significantly different after 150 days (Figure 40 and Appendix B. Table 23).

Although the content of β-carotene was found to be high in the flesh of juvenile female Tuptim tilapia fed with 50%RS and 55%RS, due to the nature of the diet treatment which already contained high β-carotene content, but the β-carotene contents found in the experimented fish eggs were not significantly different among different diet treatments. The flesh and eggs of juvenile Tuptim tilapia fed with 55%RS had high C-phycocyanin. Similarly, the high content of γ- linoleic acid in 50%RS and 55%RS had resulted in the high γ - linoleic acid in eggs of juvenile Tuptim tilapia fed with these treatments. However, the γ- linoleic acid content of the flesh from these four treatments was not significantly different (Figure 38 - 40).

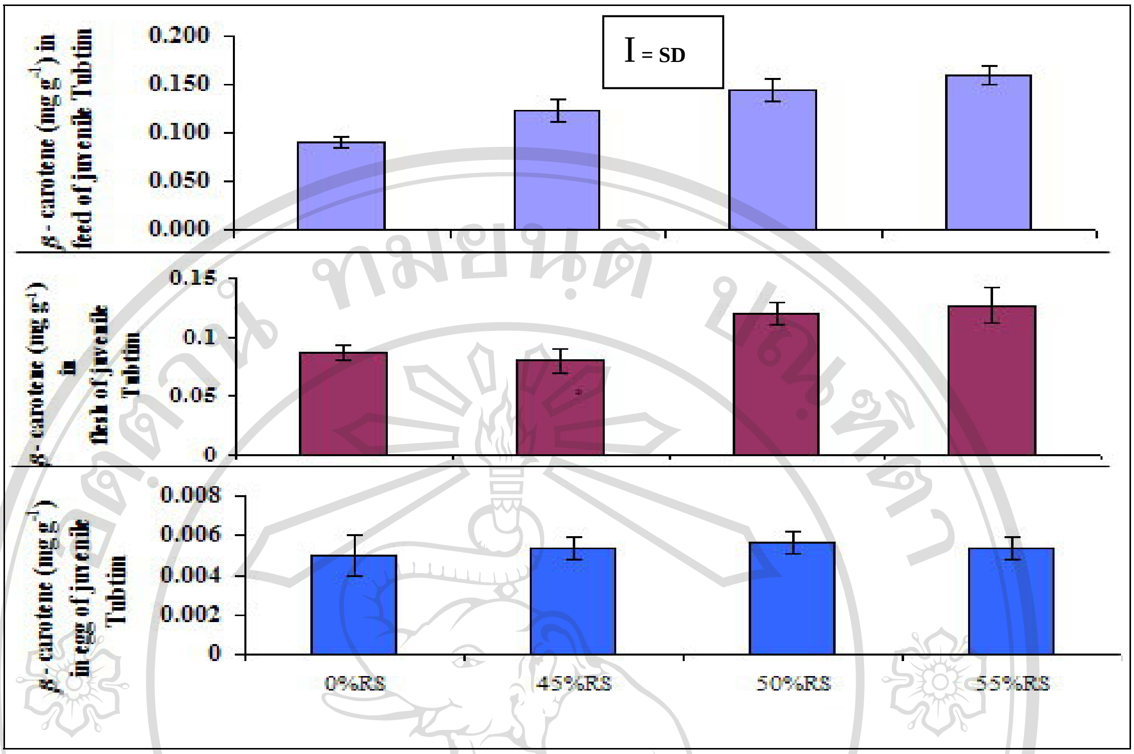

Figure 38 The analysis of β-carotene in feed, flesh and eggs of juvenile Tuptim tilapia using raw *S. platensis* culturing for 150 days

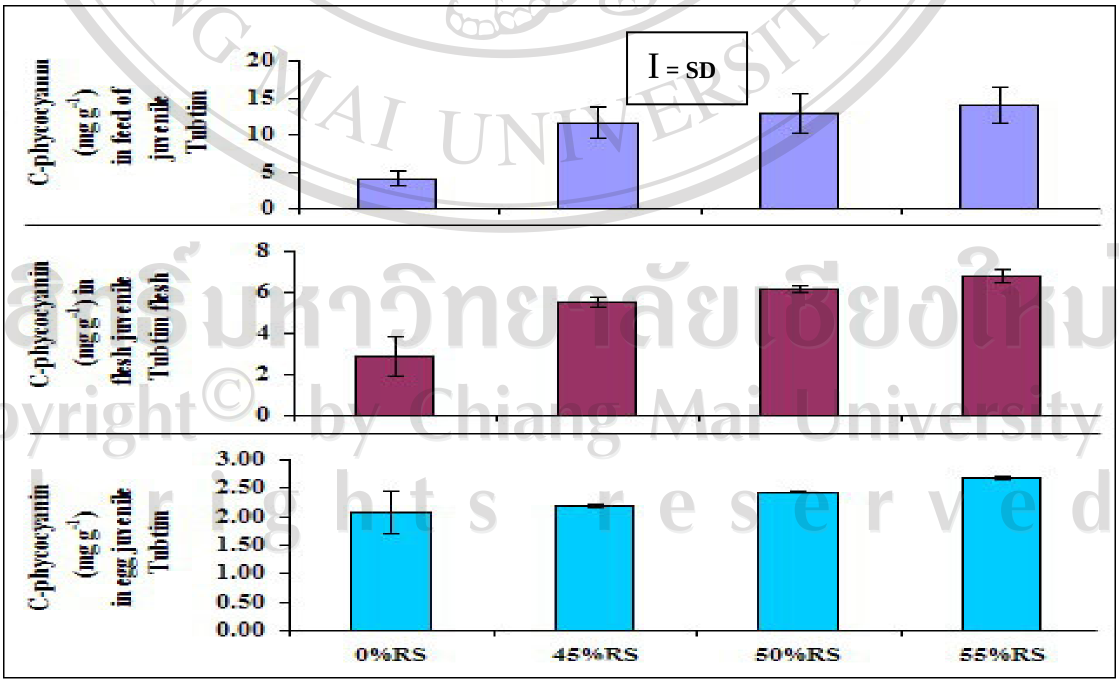

Figure 39 The analysis of C-phycocyanin in feed, flesh and eggs of juvenile Tuptim tilapia using raw *S. platensis* cultured for 150 days

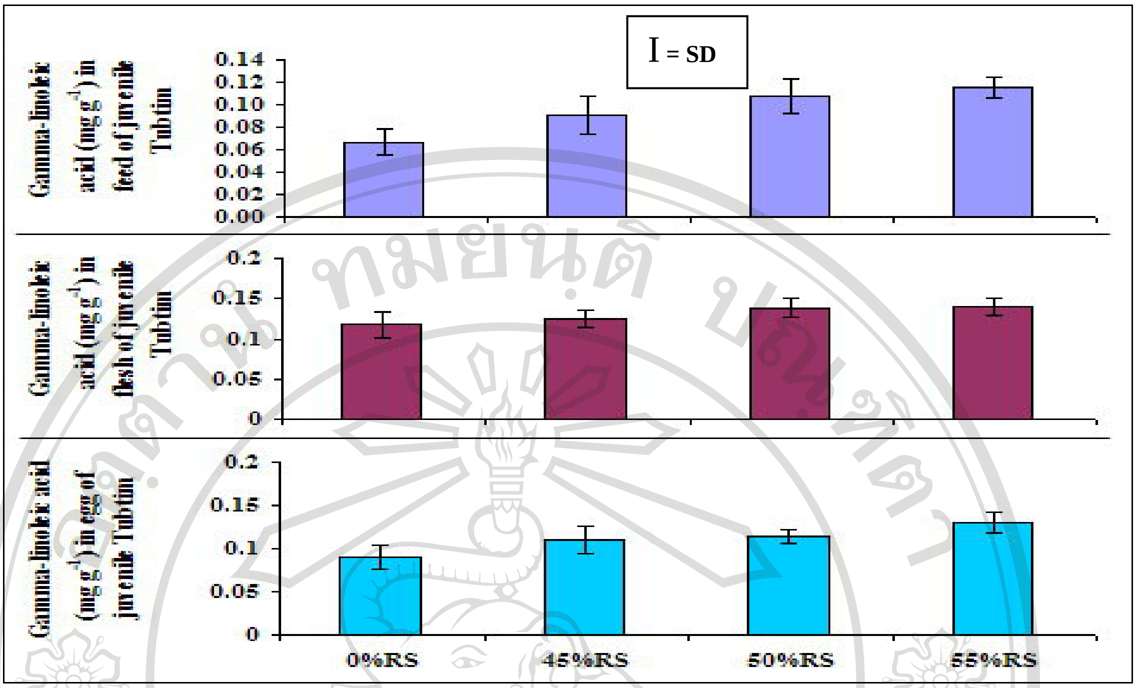

Figure 40 The analysis of γ- linoleic acid in feed, flesh and eggs of juvenile Tuptim tilapia using raw *S. platensis* cultured for 150 days

4.2.2.5 Production variable cost and nutritional value in feed, flesh and eggs of juvenile Tuptim tilapia

Protein

The protein of feed of juvenile Tuptim tilapia from all experiments were non-significantly different (Figure 41 and Appendix B. Table 24). The protein of flesh and eggs of juvenile Tuptim tilapia with 55%RS was significantly higher than that of 50%RS, 45%RS and 0%RS (control group), with $p < 0.05$ (Figure 41 and Appendix B. Table 25).

Carbohydrate or NFE

Carbohydrate of feed juvenile Tuptim tilapia with 45%RS, 50%RS and 55%RS were significantly higher than 0%RS with $p < 0.05$ (Figure 42 and Appendix B. Table 24). The carbohydrate of flesh Tuptim tilapia with all experiments were non significantly from being cultured for 150 days. The carbohydrate of eggs juveniles Tuptim tilapia with 55%RS was significantly higher than those 50%RS, 45%RS and 0%RS, respectively, with $p<0.05$ from being cultured for 150 days (Figure 42 Appendix B. Table 25).

Fat

The fat of feed juveniles Tuptim tilapia with 0%RS was significantly higher than those of 55%RS, 45%RS and 50%RS with $p<0.05$ (Figure 43 and Appendix B. Table 24). The fat of flesh of juvenile Tuptim tilapia with 45%RS was significantly higher than those of 50%RS, 0%RS and 55% RS with $p<0.05$ from being cultured for 150 days. The fat of eggs of juvenile Tuptim tilapia with 0%RS, 45%RS and 50%RS were significantly higher than those of 55%RS, with $p< 0.05$ after being cultured for 150 days (Figure 43 and Appendix B. Table 25).

Fiber

The fiber of feed juvenile Tuptim tilapia with 0%RS was significantly higher than those of 55%RS, 45%RS and 50%RS with $p < 0.05$ (Figure 44 and Appendix B. Table 24). The fiber of flesh and eggs of juvenile Tubtim tilapia from all experiments were non-significantly different (Figure 44 and Appendix B. Table 25**).**

Moisture

The moisture of feed juvenile Tuptim tilapia with 45%RS was significantly higher than those of 55%RS, 50%RS and 0%RS with $p<0.05$ (Figure 45 and Appendix B. Table 24). The moisture of meat and eggs of juvenile Tuptim tilapia from all experiments were non-significantly different (Figure 45and Appendix B. Table 25**).**

Ash

The ash of feed juvenile Tuptim tilapia with 0% RS was significantly higher than those of 55%RS, 50%RS and 45%RS with p< 0.05 (Figure 46 and Appendix B. Table 24). The ash of meat of juvenile tuptim tilapia from all experiments were non-significantly different (Figure 46 and Appendix B. Table 25**).** The ash of eggs of juveniles Tuptim tilapia with 0%RS and 45%RS were significantly higher than those 55%RS and 50%RS, with p< 0.05 after cultured for 150 days (Figure 46 and Appendix B. Table 25).

Production variable cost

The production variable cost of juvenile Tuptim tilapia from all experiments were non-significantly different (Appendix B. Table 24).

Summary of the diet treatments (50%RS and 55%RS) in culturing female juvenile Tuptim tilapia had the result of high protein in the flesh and eggs of the fish. These two diet treatments had significantly high carbohydrate in the eggs but not in the flesh of the fish. These treatments gained the lower fat in flesh and eggs of the fish. In addition, the production variable costs of juvenile Tuptim tilapia from all experiments were not significantly different.

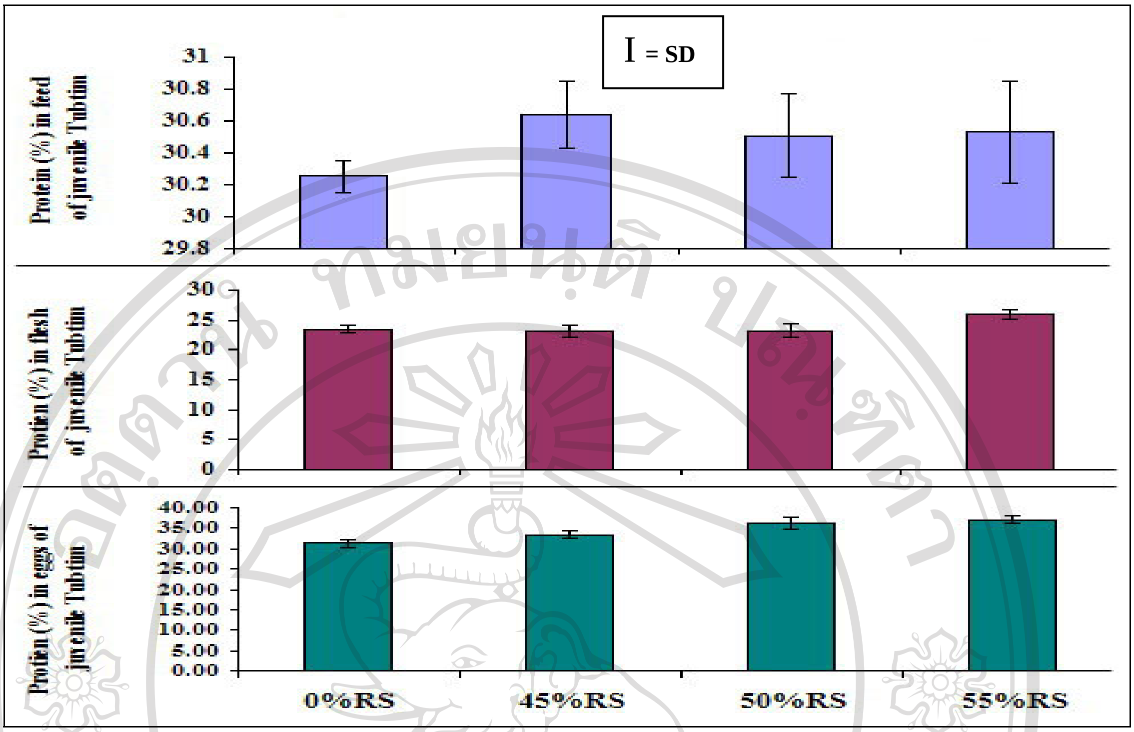

Figure 41 The analysis of percent protein in feed, flesh and eggs of juvenile Tuptim tilapia using raw *S. platensis* feed cultured in the earthen pond for 150 days

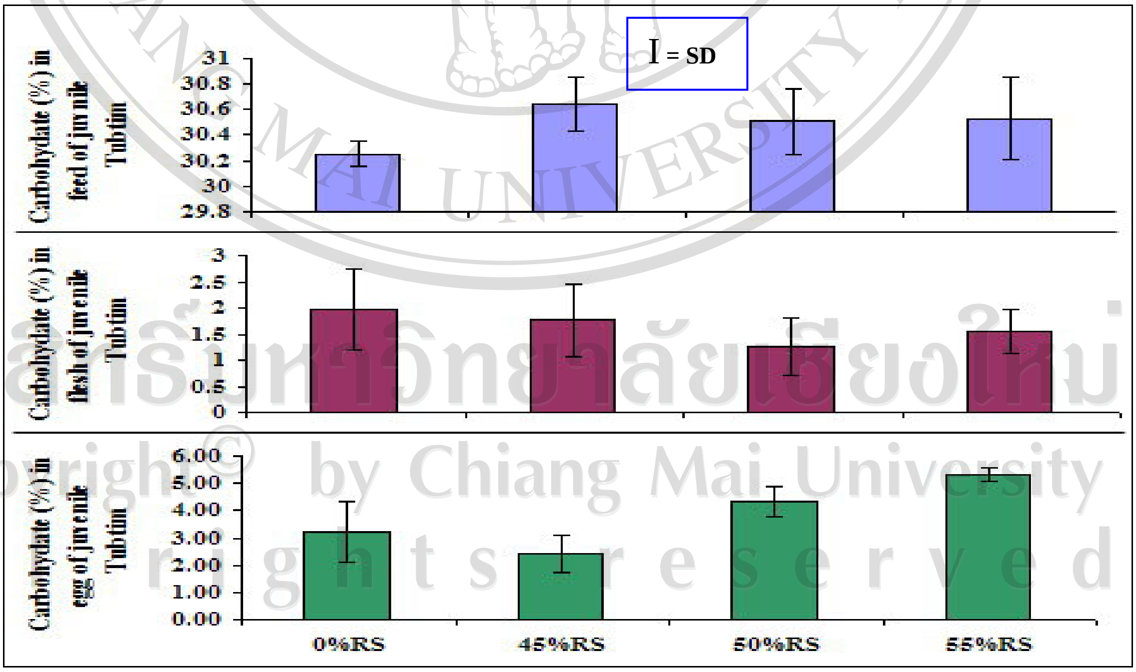

Figure 42 The analysis of percent carbohydrate in feed, flesh and eggs of juvenile Tuptim tilapia using raw *S. platensis* feed cultured in the earthen pond for 150 days

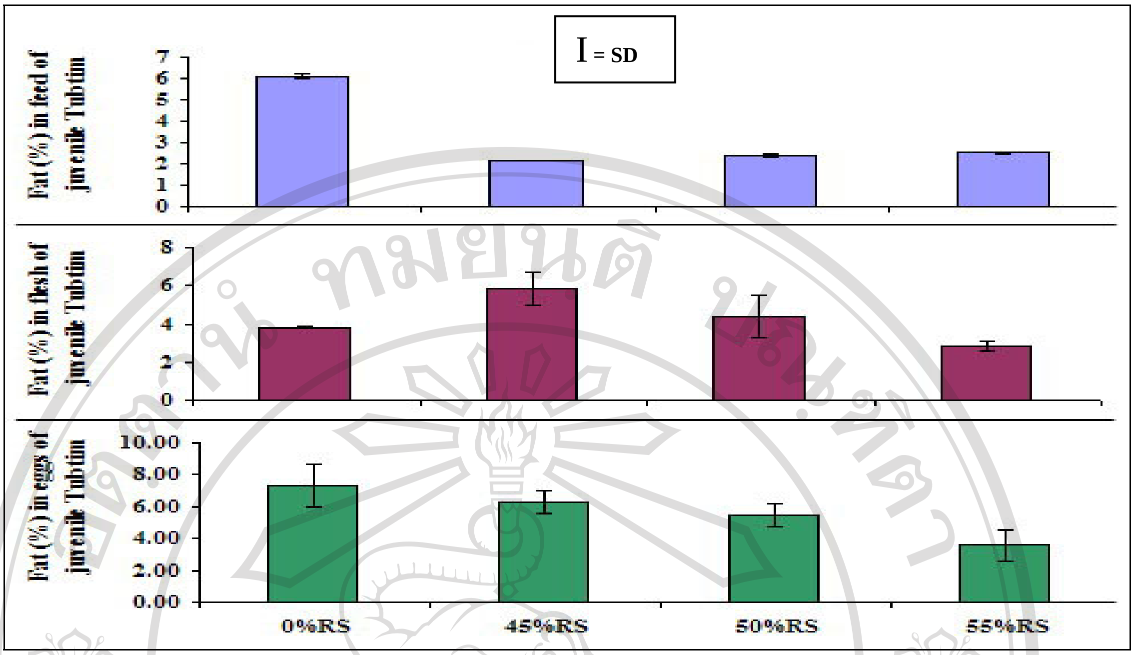

Figure 43 The analysis of percent fat in feed, flesh and eggs of juvenile Tuptim tilapia using raw *S. platensis* feed cultured in the earthen pond for 150 days

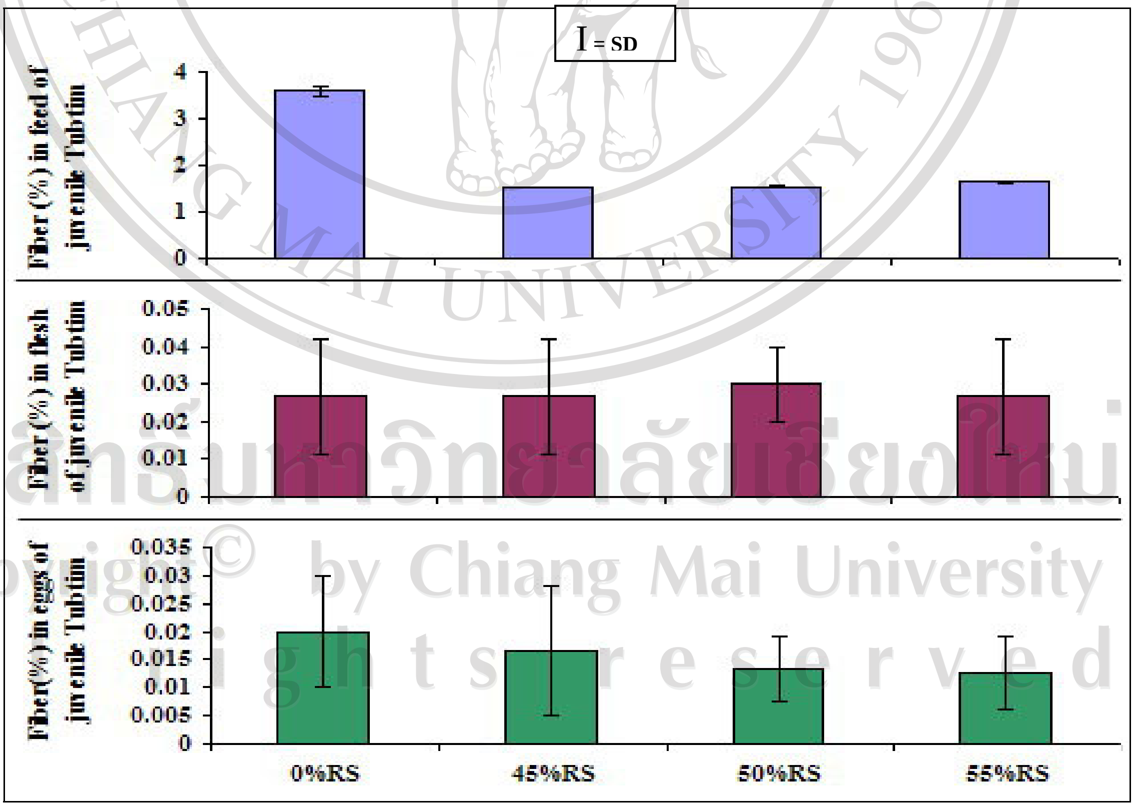

Figure 44 The analysis of percent fiber in feed, flesh and eggs of juvenile Tuptim tilapia using raw *S. platensis* feed cultured in the earthen pond for 150 days

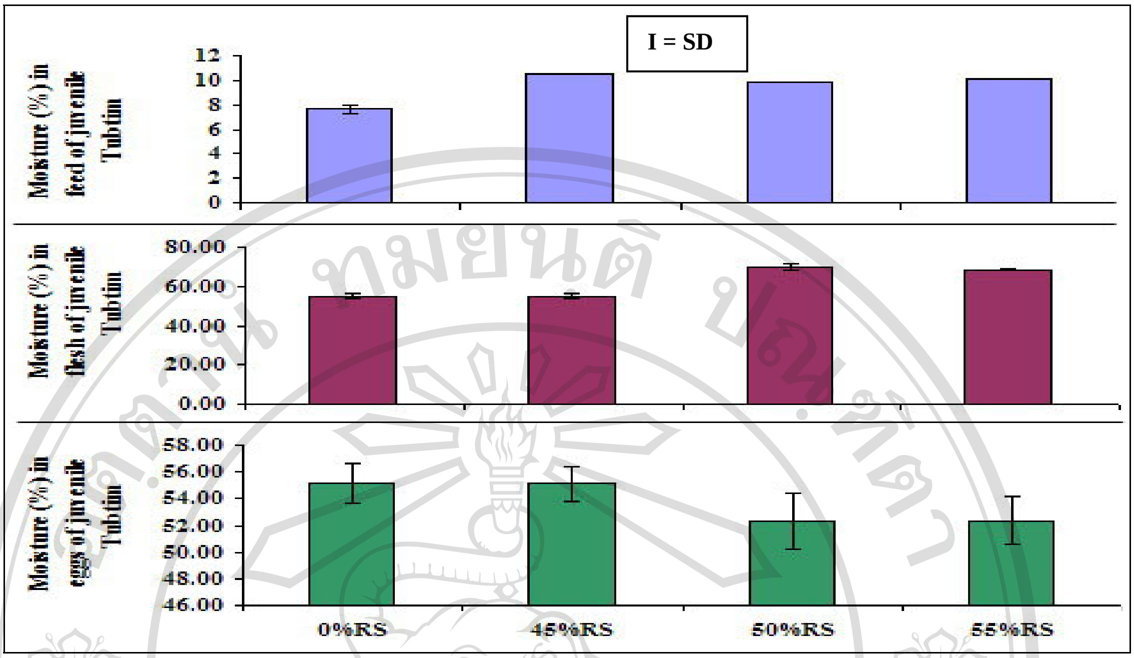

Figure 45 The analysis of percent moisture in feed, flesh and eggs of juvenile Tuptim tilapia using raw *S. platensis* feed cultured in the earthen pond for 150 days

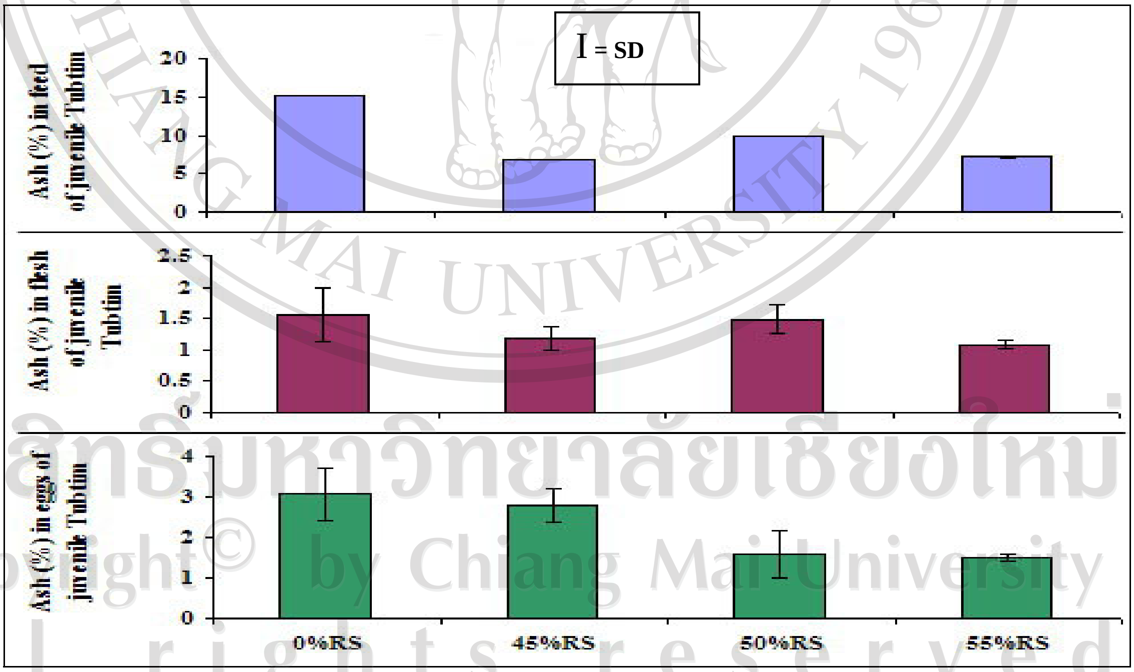

Figure 46 The analysis of percent ash in feed, flesh and eggs of juvenile Tuptim tilapia using raw *S. platensis* feed cultured in the earthen pond for 150 days

4.2.2.6 Gonadosomatic index; GSI %) of male and female juvenile Tuptim tilapia cultured in the earthen pond for 150 days

The GSI from male juvenile Tuptim tilapia with 55%RS, 50%RS and 45%RS were significantly higher than that of 0%RS with p< 0.05 after cultured for 150 days. The GSI from female juvenile Tuptim tilapia with 55%RS, 50%RS were significantly higher than those of 45%RS and 0%RS with p< 0.05 after cultured for 150 days (Figure 47 and Appendix B. Table 26).

The results showed that the diet treatments of 45%RS, 50%RS and 55%RS indicated high gonadosomatic index (GSI %).

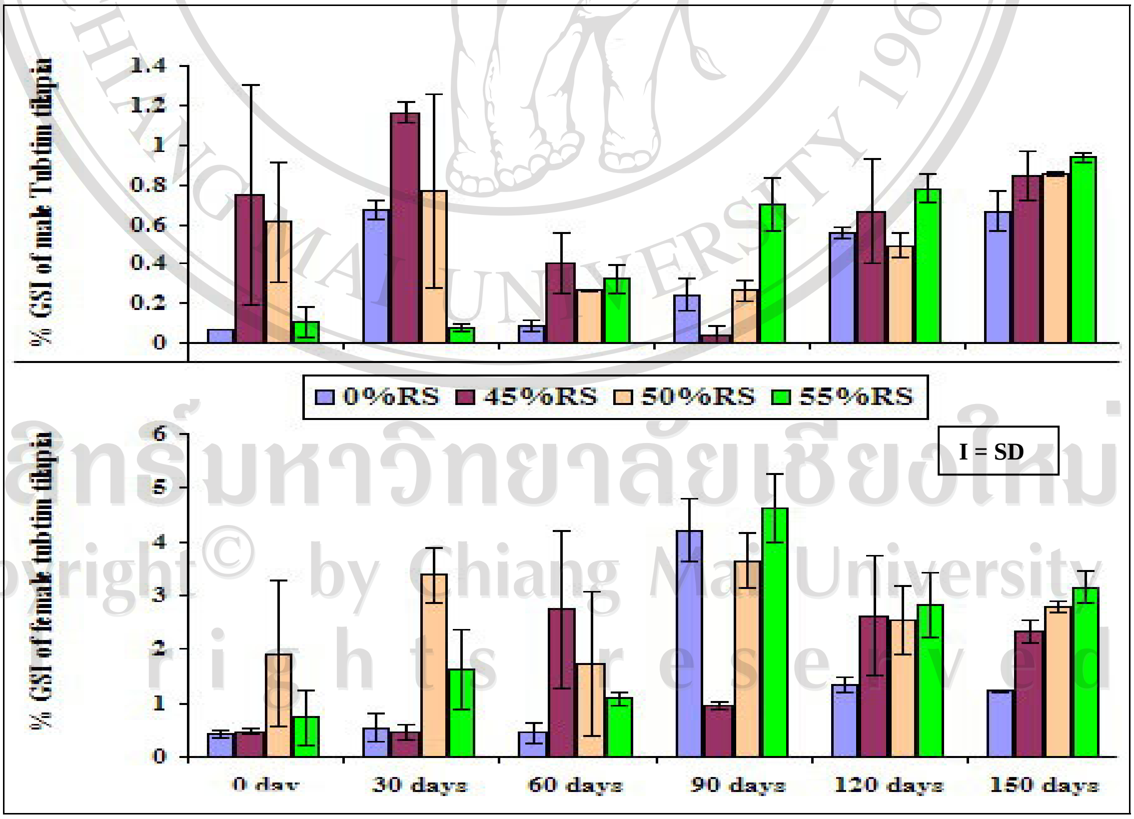

Figure 47 The analysis of gonadosomatic index (GSI; %) male and female juvenile Tuptim tilapia cultured in the earthen pond for 150 days.

4.2.2.7 Analyses for physico-chemical qualities of water where in juvenile tuptim tilapia cultured in the earthen pond

Water temperature and pH

Water temperature in the juvenile Tuptim tilapia culturing earthen pond with 45%RS was significantly higher than those of 0%RS control), 50%RS and 55%RS with $p< 0.05$ at day 0 (Appendix B. Table 27). All four experimental treatments of juvenile Tubtim cultured in earthen ponds had shown pH values of non-significant different after cultured for 150 days (Figure 48 and Appendix B. Table 27).

Alkalinity (mg L^{-1}) and total dissolved solid (TDS; mg L^{-1})

The alkalinity in juvenile Tuptim tilapia culturing earthen pond with 55% RS, 50% RS and 0% RS were significantly higher than that of 45%RS with $p< 0.05$ cultured for 150 days. The TDS in juvenile Tuptim tilapia culturing earth pond with 50%RS and 55%RS were significantly higher than those of 45%RS and 0%RS with $p< 0.05$ after cultured for 150 day (Figure 48 and Appendix B. Table 27).

Conductivity (μs cm^{-1}) and Dissolved Oxygen (DO; mg L^{-1})

The conductivity in juvenile Tuptim tilapia culturing earthen pond with 45%RS, 50%RS and 55%RS were significantly higher than that of 0%RS with $p< 0.05$ after cultured at days 120 and 150. The DO in the juvenile Tuptim tilapia culturing earthen pond with 55%RS, 50%RS and 0%RS were significantly higher than that of 45%RS after cultured at days 120 and 150 (Figure 48 and Appendix B. Table 27).

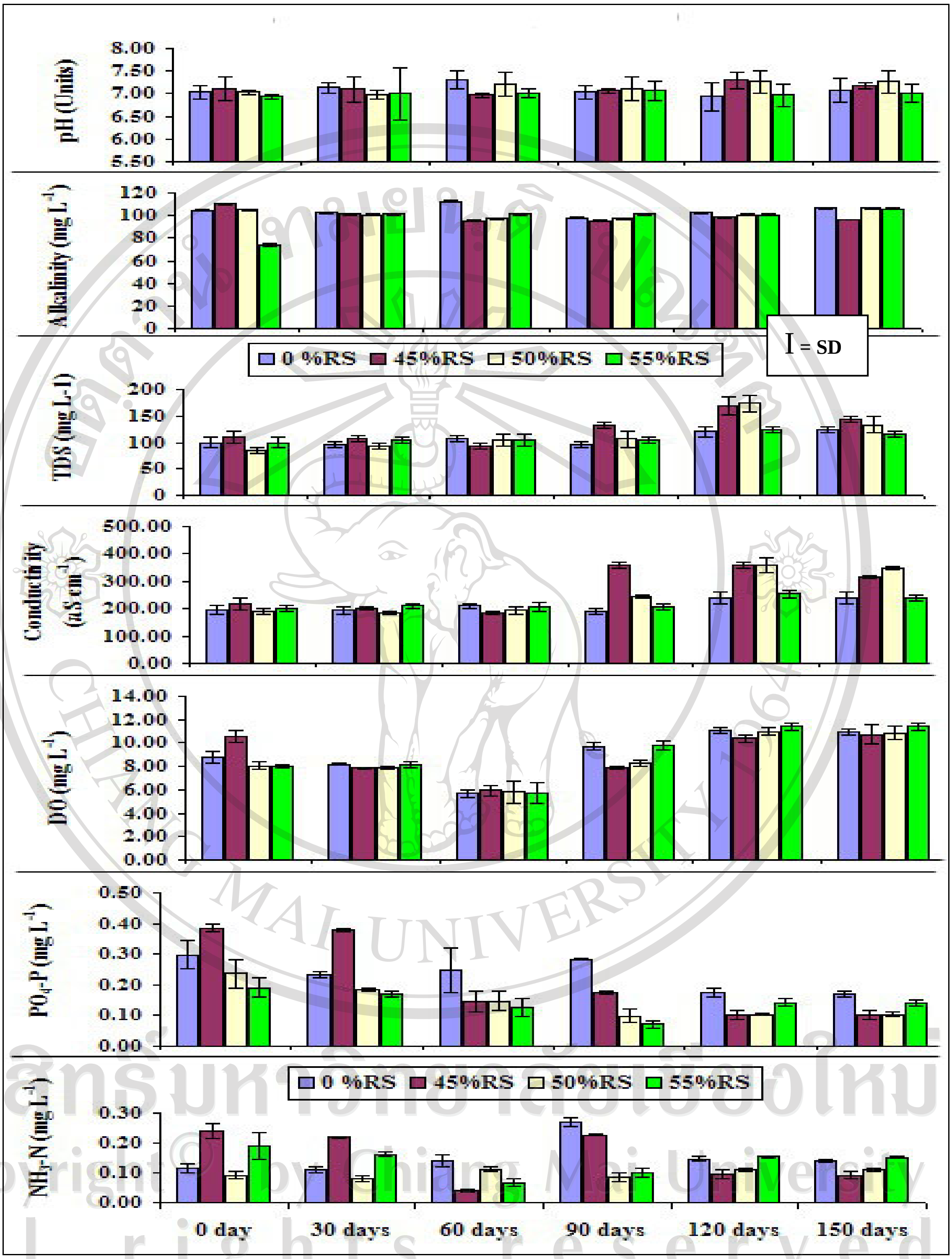

Figure 48 The analysis of pH, alkalinity, conductivity, TDS, DO, PO_4-P and NH_3-N in juvenile Tuptim tilapia culturing pond using raw *S. platensis* feed for 150 days.

Ammonia - nitrogen (NH_3-N; mg L^{-1}) and orthophosphates - phosphorus (PO_4-P; mg L^{-1})

The NH_3-N in juvenile Tuptim tilapia culturing earthen pond with 0% RS and 55%RS were significantly higher than those of 50%RS and 45%RS with $p < 0.05$ after cultured for 150 days. The PO_4-P in juvenile Tuptim tilapia culturing earthen pond with 0%RS was significantly higher than those of 45%RS, 55%RS and 50%RS withp< 0.05 after cultured for 150 days (Figure 48 and Appendix B. Table 27).

For the analyses of physico-chemical water quality of the culturing Tuptim tilapia in earthen ponds, it indicated the water temperature of 20.83–28.67 ^{o}C, water pH of 6.93–7.30, alkalinity of 95.00–107.00 mg g^{-1}, TDS of 93.00–173 mg g^{-1}, conductivity of 183.00–360.00 μS cm^{-1}, DO of 5.67–11.40 mg g^{-1}, PO_4-P of 0.10–0.30 mg g^{-1} and NH_3-N of 0.04–0.27 mg g^{-1}. It could be concluded that the water quality of the culturing ponds of Tubtim tilapia was within the standard values of surface water (Tave, 1990).