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Growth and Nutritional Value of a Tropical Green Alga, *Ankistrodesmus convolutus* Corda, in Agro-industrial Effluents

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ABSTRAK

Penggunaan efluen agro-industri bagi pengkulturan mikroalga telah dikaji dengan menggunakan mikroalga air tawar tropikal, Ankistrodesmus convolutus *yang dikultur di dalam pelbagai cairan efluen getah lateks pekat (LCRE), getah piawaian Malaysia (SMRE) dan kelapa sawit tercerna (POMED).* Ankistrodesmus convolutus *yang dikultur di dalam 40% dan 60% LCRE, 60% SMRE dan 10% POMED telah meningkatkan kadar pertumbuhan spesifik (dari segi bilangan sel dan klorofil a) yang jauh lebih tinggi (P < 0.05) dibandingkan dengan alga yang tumbuh di dalam efluen lain dan baja tak organik (N:P:K = 1:1:0.5) sebagai kawalan. Jumlah biojisim yang dikultur dalam 60% LCRE, 60% SMRE dan 10% POMED adalah lebih tinggi (P < 0.05) dibandingkan alga yang tumbuh di dalam efluen lain dan kawalan.* Ankistrodesmus convolutus *yang dikultur di dalam 40% dan 60% LCRE, 60% SMRE dan 10% POMED mempunyai protein mentah dan lipid yang lebih tinggi (P < 0.05) dibandingkan dengan mikroalga yang tumbuh dalam medium lain. Kebanyakan asid amino perlu (EAA) adalah lebih tinggi dalam alga yang tumbuh di dalam 60% LCRE, 60% SMRE, dibandingkan dengan efluen lain.* Ankistrodesmus convolutus *yang dikultur di dalam 10% POMED mengandungi jumlah EAA yang tinggi kecuali treonin dan tirosin dibandingkan dengan alga yang dikultur dalam medium POMED lain dan kawalan.* Ankistrodesmus convolutus *yang dikultur di dalam 40% dan 60% LCRE mengandungi jumlah asid lemak poli-tak-tepu (PUFA), C18 dan C20, yang lebih tinggi (P < 0.05) daripada alga dalam medium SMRE lain dan kawalan, kecuali asid eikosadienoik (20:2n-11). Corak PUFA yang sama direkodkan dalam alga yang dikultur dalam 60% SMRE kecuali asid eikosatrienoik (20:3n-6) dan asid arakidonik (20:4n-6).* Ankistrodesmus convolutus *yang dikultur di dalam 10% POMED mengandungi PUFA seperti asid linoleik (18:2n-6), asid linolenik (18:3n-3) dan asid arakidonik yang lebih tinggi dibandingkan dengan alga yang tumbuh dalam medium POMED lain dan kawalan. Kajian ini menunjukkan* A. convolutus *yang dikultur dalam 40% - 60% getah dan 10% POMED mempunyai nilai pemakanan yang lebih tinggi dibandingkan dengan alga yang dikultur dalam medium lain.*

ABSTRACT

Use of agro-industrial effluents for microalgal culture was investigated using a tropical freshwater green alga, Ankistrodesmus convolutus *cultured in various dilutions of latex concentrate effluent (LCRE), standard Malaysian rubber effluent (SMRE) and digested palm oil mill effluent (POMED).* Ankistrodesmus convolutus *grown in 40% and 60% LCRE, 60% SMRE and 10% POMED showed significantly higher (P < 0.05) specific*

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growth rate in terms of cell number and chlorophyll a than that grown in other effluent media and inorganic fertiliser (N:P:K = 1:1:0.5) as control. Total biomass of this microalgae grown in 60% LCRE, 60% SMRE and 10% POMED was significantly higher ($P < 0.05$) than that cultured in other effluent media and the control. Ankistrodesmus convolutus *cultured in 40% and 60% LCRE, 60% SMRE and 10% POMED showed significantly ($P < 0.05$) higher amount of crude protein and lipid than that grown in other effluent media and the control. Significantly higher ($P < 0.05$) amount of most of the essential amino acids (EAAs) except a few were found in* A. convolutus *cultured in 60% LCRE and 60% SMRE than that grown in other effluent media and control.* Ankistrodesmus convolutus *cultured in 10% POMED resulted in significantly higher ($P < 0.05$) amount of all the EAAs except threonine and tyrosine that were grown in other POMED media and control.* Ankistrodesmus convolutus *grown in 40% and 60% LCRE, contained significantly higher ($P < 0.05$) amounts of all the C18 and C20 polyunsaturated fatty acids (PUFAs) than that cultured in other SMRE media and control, except eicosadienoic acid (20: 2n-11). A similar trend of PUFAs was recorded in* A. convolutus *cultured in 60% SMRE except eicosatrienoic acid (20:3n-6) and arachidonic acid (20:4n-6). It was found that* A. convolutus *contained significantly ($P < 0.05$) higher amount of PUFAs such as linoleic acid (18:2n-6), linolenic acid (18:3n-3) and arachidonic acid (20:4n-6) when grown in 10% POMED than that cultured in other POMED media and control. This study showed that* A. convolutus *grown in 40-60% rubber and 10% POMED has higher nutritional value that that cultured in other effluent media and inogranic fertilizer.*

INTRODUCTION

Microalgae biosynthesize essential nutrients like proteins, lipids, essential amino acids (EAAs), polyunsaturated fatty acids (PUFAs) and essential minerals (Chu *et al.* 1991; 1995; Habib *et al.* 1997; Vazhappilly and Chen 1998) when grown in appropriate media. Most of the freshwater microalgae contain high amount of C1‘8 PUFAs and low amount of C20 PUFAs, but C22 PUFAs are very rarely found (Ben-Amotz *et al.* 1985; Geldenhuys *et al.* 1988; Chu *et al.* 1994) when grown in media without manipulation of nutrients. On the other hand, microalgae can biosynthesize high amount of carotenoids, some EAAs, C18, C20 and C22 PUFAs if the culture media contain one or more electrolytes and nutrients such as peptides, hydrocarbons, sucrose, glucose, short carbon chain compounds, minerals (Tan and Johns 1991; Laliberte and de la Noüe 1993; Barclay *et al.* 1994; Combres *et al.* 1994; Vazhappilly and Chen 1998).

The agroindustrial effluents such as rubber and palm oil mill effluents contain high organic matter, as indicated by high chemical oxygen demand (COD) (Phang 1987; Isa *et al.* 1988; Habib *et al.* 1998). They are also rich in nutrients including protein, lipid, carbohydrate and micronutrients as amino acids, fatty acids and minerals (Hwang 1978; Okiy 1987; Isa *et al.* 1993; Kekwick 1997; Habib *et al.* 1998). In addition, these effluents can be important sources of inorganic nutrients and short carbon chain compounds like those found in heterotrophic culture media (Isa *et al.* 1988; Phang 1990). The aim of this work was to illustrate the recovery of valuable inorganic and organic nutrients in agro-industrial waste water through biosynthesis by microalgae.

MATERIALS AND METHODS

The raw latex concentrate rubber effluent (LCRE) from Atherton Estate factory, Atherton, Negeri Sembilan, standard Malaysian rubber effluent (SMRE) from Kilang Getah MARDEC Berhad, Selangor D.E. and palm oil mill effluent from Golden Hope Mill, Pulau Carey Island, Selangor D. E. were collected and carried to the laboratory. All the effluents were collected at the discharge point from mills to the drain. The raw LCRE and SMRE were aerated in tanks for 5 days to break down the sulphides overnight, and to increase pH value to approximately 7.0. On the sixth day, the effluents were diluted into four different concentrations i.e. 20%, 40%, 60% and 80% of 100% effluent. The raw POME was digested and organic nutrients were broken into simpler forms by bacteria in aerobic conditions using continuous air flow. After 16 days, the brownish coloured liquid portion was formed in the upper layers. The upper layer of digested POME (POMED) was then diluted into four concentrations i.e. 5%, 10%, 20%, 30% of 100% POMED.

Ankistrodesmus convolutus (isolate no. 101) was innoculated in 5 different concentrations of LCRE and SMRE including 100% LCRE and SMRE, four different concentrations of digested Palm Oil Mill Effluents (POMED) and in inorganic fertilizer, N:P:K (1.0: 1.0: 0.50) as control in 70 1 tanks in the hatchery (Table 1). Each treatment had three replicates. The pH, optical density, chlorophyll *a*, and specific growth rates in terms of cell numbers and chlorophyll *a* were recorded (Clesceri *et al.* 1989). Total carotene, observed yield, proximate composition, amino acids and fatty acids of the microalga were analyzed from samples collected before reaching the stationary phase of the growth. The microalge were cleaned with deionized water and centrifuged repeatedly. The cleaned precipitate of alga was freeze dried at – 50⁰C under 150 millitorr pressure for 1.5 days. The freeze-dried samples were preserved at –80⁰C for biochemical analyses.

Proximate composition of the samples was determined according to the methods of Horwits (1984). The deproteinized samples were hydrolyzed with 2.0 ml 4.0 M methanesulfonic acid containing 0.20% tryptamine. The amino acids were determined from the peaks which appeared after injection of 10μl filtered hydrolyzed samples onto a single column Waters HPLC 501 (Millipore 1990) and as described by Habib *et al.* (1997b). The fatty acid methyl esters (FAME) were obtained form lipid hydrolyses of samples by refluxing at 100⁰C with 14% boron trifluoride-methanol according to the methods of Folch *et al.* (1957) as modified by Benitez (1989). The FAME of 1.0 μl were injected at 130⁰C onto a single column Shimadzu GC-14A gas chromatography unit and fatty acids were analyzed following Habib *et al.* (1997b). To compare treatment means of proteins, lipids, carbohydrates, ash, essential amino acids (EAAs) and unsaturated fatty acids (UFAs) of *A. convolutus* grown in different media, one way ANOVA was performed using SAS computer package followed by the Tukey Test (Zar 1984).

RESULTS AND DISCUSSION

Among the growth parameters, specific growth rate (SGR) of cell and chlorophyll *a*, and total biomass of *A. convolutus* cultured in 40% & 60% LCRE, 60% SMRE and 10% POMED were significantly ($p < 0.05$) higher than those of *A convolutus* grown in other effluent media and the control N:P:K fertilizer (Table 2). All the growth parameters of *A. convolutus* grown in 10% POMED were significantly ($P < 0.05$) higher than that cultured in other POMED media and the control (Table 2). High growth performances in the above mentioned media might be due to adequate micro nutrients available in the media, appropriate colour of media which permitted sufficient light to penetrate in the media and sufficient supply of filtered air to overcome carbon dioxide (Phang 1991a; Anton 1994; Johns 1994; Yusoff *et al.* 1997). However, the C:N:P ratios of different dilutions of raw LCRE and SMRE (Table 3) showed almost three times less than the recommended ratio (56.30: 8.60: 1.20 for microalgae) (Edwards *et al.* 1980) which agrees with the findings of Phang and Ong (1988).

Rubber effluent is deficient in carbon content which may be partially solved through adequate filtered air supply and light penetration (Johns 1994) as well as flow of carbon dioxide gas (Phang and Ong 1988; Geetha *et al.* 1994) into the effluent media. Values of pH of all the media at the exponential phase of *A. convolutus* growth were above 10, indicating high cell growth and production during the study. Phang (1990; 1991) also reported high growth of microalgae in agro-industrial wastes. Anton *et al.* (1994) reported that *Scenedesmus quadricauda* showed high growth rate for concentrations of palm oil mill effluent (POME) below 14% collected at a point of discharge into river three km away from the mill. In addition, Phang (1987) and Yusoff and Chan (1997) found that diluted and digested palm oil mill effluent enhanced higher growth of a green alga, *Selenastrum capricornutum*. The effluent media contained high chemical oxygen

TABLE 1
Different concentrations (%) of raw latex concentrate rubber effluent (LCRE) standard Malaysian rubber effluent (SMRE) and digested palm oil mill effluent (POMED)

Treatment	Raw LCRE (%)	Raw SMRE (%)	POMED (%)
T1	20.0	20.0	5.0
T2	40.0	40.0	10.0
T3	60.0	60.0	20.0
T4	80.0	80.0	30.0
T5	100.0	100.0	-

TABLE 2

Specific growth are (μ/day) of cell and chlorophyll *a* (Chl-*a*), total carotene and total biomass of *Ankistrodesmus convolutus* grown in different dilutions of latex concentrate rubber effluent (LCRE), standard Malaysian rubber effluent (SMRE), digested palm oil effluent (POMED), and fertilizer. Means (± SE) with different superscripts in each row indicate significant differences (P<0.005), *mg/g. **mg/1

Parameters	20%LCRE	40%LCRE	60%LCRE	80%LCRE	100%LCRE	Fertilizer
SGR of Cell	0.32±0.03^{b}	0.38±0.03^{a}	0.40±0.03^{a}	0.30±0.03^{bc}	0.27±0.02^{c}	0.33±0.02^{b}
SGR of Chl-*a*	0.31±0.02^{b}	0.36±0.03^{a}	0.38±0.03^{a}	0.28±0.02^{c}	0.27±0.02^{c}	0.33±0.02^{b}
Total carotene*	6.29±0.05^{b}	6.45±0.05^{a}	6.65±0.05^{a}	6.46±0.05^{a}	6.37±0.05^{a}	5.53±0.04^{b}
Total biomass (Chl-*a* x 67)**	509.20 ±8.43^{f}	791.94 ±11.75^{b}	925.94 ±13.95^{a}	616.40 ±9.45^{c}	519.25 ±8.77^{e}	559.45 ±9.35
	20%SMRE	40%SMRE	60%SMRE	80%SMRE	100%SMRE	N:P:K
SGR of Cell	0.30±0.02^{b}	0.32±0.03^{b}	0.39±0.03^{a}	0.30±0.03^{b}	0.26±0.02^{c}	0.33±0.02^{b}
SGR of Chl-*a*	0.31±0.02^{b}	0.33±0.03^{b}	0.37±0.03^{a}	0.27±0.02^{b}	0.26±0.02^{b}	0.33±0.02^{a}
Total carotene*	6.35±0.05^{a}	6.55±0.05^{a}	6.73±0.05^{a}	6.53±0.05^{a}	6.45±0.05^{a}	5.55±0.04^{b}
Total biomass (Chl-*a* x 67)**	475.70 ±6.15^{f}	787.25 ±10.75^{b}	907.85 ±13.92^{a}	600.32 ±9.21^{c}	506.52 ±7.55^{e}	562.13 ±9.15^{d}
	5% POMED	10% POMED	20% POMED	30% POMED		Fertilizer
SGR of Cell	0.29±0.02^{bc}	0.36±0.03^{a}	0.26±0.02^{c}	0.22±0.02^{d}	-	0.32±0.02^{b}
SGR of Chl-*a*	0.33±0.03^{b}	0.38±0.03^{a}	0.27±0.02^{c}	0.21±0.02^{d}	-	0.33±0.02^{b}
Total carotene*	6.45±0.05^{b}	6.95±0.06^{a}	6.34±0.06^{b}	6.25±0.05^{b}	-	5.57±0.04^{c}
Total biomass (Chl-*a* x 67)**	509.20 ±8.66^{e}	924.60 ±12.96^{a}	800.65 ±11.82^{b}	769.83 ±9.62^{c}	-	564.81 ±9.22^{d}

demand, total solids, total suspended solids, total N, ortho-phosphate (Table 3) which proved its potential as a source of inorganic nutrients for good growth of microalgae and other aquatic microplants. Phang (1991), Geetha *et al.* (1994) and Habib *et al.* (1998) reported that rubber and palm oil effluents media were rich in inogranic nutrients, COD, total N, broken protein, lipids, carbohydrates and minerals.

Both protein and lipids were significantly ($P < 0.05$) higher in *A. convolutus* cultured in 40% and 60% LCRE, 60% SMRE and 10% POMED than other media (Table 4). Carbohydrate content of *A. convolutus* grown in N:P:K, and 20% LCRE, 20% and 40% SMRE, and 5% POMED were significantly ($P < 0.05$) higher than that of *A. convolutus* cultured in other effluent media. It was observed that both protein and carbohydrates of *A. convolutus* were higher than the lipid contents except in 10% POMED. The high growth and nutritional contents of the alga were probably due to the availability of essential nutrients in the effluents (Combres *et al.* 1994; Yusoff *et al.* 1996).

Ankistrodesmus convolutus biosynthesized all the 11 essential amino acids (EAAs) when cultured in effluent media. It contained significantly higher ($P < 0.05$) amount of EAAs viz. Histidine, threonine, tyrosine, phenylalaniene, valine, methionie, lysine and tryptophan cultured in 60% LCRE than that grown in other LCRE media and control (Table 5). Significantly ($P < 0.05$) higher amounts of arginie, methionine, leucine, lysine and tryptophan were biosynthesized when grown in 60% SMRE than grown in other SMRE media and control (Table 6). Furthermore, *A. convolutus* contained significantly ($P < 0.05$) higher amount of all the EAAs except threonine and tyrosine when grown in 10% POMED than that grown in other POMED media and control (Table 7). Combres *et al.* (1964) and Laliberte and de la Noüe (1993) reported that micronutrients in culture media including nitrogenous compounds, phosphorus compounds, minor nutrients are channeled into protein, carbon compounds and essential nutrients like amino acids. It was found that the nonessential amino acids such as aspartic acid, glutamic acid, serine, glycine, alanine and proline had considerable contribution to total amino acids of *A. convolutus* cultured in all the media including the control.

Ankistrodesmus convolutus contained most of the polyunsaturated fatty acids (PUFAs) of C18 and C20 in considerable amounts when grown in all the effluent media compared to that

TABLE 3
Average chemical contents (mg/L except pH) of different dilutions of raw latex concentrate rubber effluent (LCRE), standard Malaysian rubber effluent (SMRE) and digested palm oil mill effluent (POMED)

Parameters	20%LCRE	40%LCRE	60%LCRE	80%LCRE	100%LCRE
pH	7.10 ± 0.10	6.90 ± 0.10	6.80 ± 0.10	6.60 ± 0.15	4.45 ± 0.09
Dis. O_2	3.90 ± 0.10	3.75 ± 0.09	3.60 ± 0.10	3.50 ± 0.15	0
COD	1505.24 ± 2.67	3062.49 ± 3.66	4605.73 ± 5.54	6269 ± 8.12	7906.22 ± 12.44
TS	1256.40 ± 2.87	2556.80 ± 3.66	3622.20 ± 5.15	4842.60 ± 7.22	6562.72 ± 10.35
TSS	1175.08 ± 1.12	2306.12 ± 2.08	3588.17 ± 3.11	4774.23 ± 4.15	5945.29 ± 6.23
Total N	231.03 ± 1.08	472.06 ± 2.05	668.09 ± 2.88	934.12 ± 3.67	1205.15 ± 5.28
NH_3-N	92.33 ± 0.52	179.64 ± 0.86	282.45 ± 1.15	377.33 ± 1.90	496.66 ± 2.44
PO_4-P	32.95 ± 0.44	70.89 ± 0.62	102.84 ± 0.79	142.78 ± 0.79	189.73 ± 1.68
C:N:P ratio	15.70:9.0:1.0	16.20:9.33:1.0	16.80:9.24:1.0	16.47:9.19:1.0	15.63:8.97:1.0
Parameters	**20%SMRE**	**40% SMRE**	**60% SMRE**	**80% SMRE**	**100% SMRE**
pH	6.90 ± 0.10	6.70 ± 0.10	6.50 ± 0.10	5.60 ± 0.15	4.35 ± 0.12
Dis.O_2	2.85 ± 0.10	2.75 ± 0.09	2.60 ± 0.10	2.50 ± 0.15	0
COD	695.55± 2.56	1266.52 ± 4.54	1985.33 ± 4.54	2724.12 ± 5.66	3506.08 ± 8.66
TS	505.47 ± 1.04	1033.45 ± 2.55	1617.41 ± 3.25	2165.78 ± 6.88	2862.35 ± 7.55
TSS	466.05 ± 0.83	916.09 ± 1.14	1464.14 ± 2.07	2036.17 ± 3.55	2550.22 ± 5.38
Total N	176.08 ± 1.22	345.15 ± 1.68	542.22 ± 2.15	716.30 ± 2.74	985.37 ± 3.66
NH_3-N	55.20 ± 0.45	97.40 ± 0.66	155.60 ± 0.75	218.92 ± 0.90	296.15 ± 1.45
PO_4-P	22.02 ± 0.28	45.03 ± 0.46	68.05 ± 0.67	87.07 ± 0.83	125.08 ± 1.07
C:N:P ratio	11.85:10.50:1.0	10.55:9.83:1.0	10.94:10.26:1.0	11.73:10.74:1.0	10.51:10.25:1.0
Parameters	**5% POMED**	**10% POMED**	**20% POMED**	**30% POMED**	**100%POMED**
pH	7.10 ± 0.10	6.90 ± 0.10	6.80 ± 0.10	6.60 ± 0.10	6.40 ± 0.10
Dis.O_2	3.90 ± 0.10	3.75 ± 0.09	3.60 ± 0.10	3.50 ± 0. 10	2.10 ± 0.10
COD	1042.65. ± 12.75	2179.50 ± 25.55	4245.45 ± 75.66	6458.85 ± 65.12	21842.40 ± 30.42
TS	443.15 ± 5.04	975.72± 8.05	1926.02 ± 11.25	2844.32 ± 15.07	10128.80 ± 85.50
TSS	252.25 ± 3.03	524.52 ± 4.04	959.65 ± 6.07	1645.25 ± 8.12	5443.50 ± 25.90
Total N	58.52 ± 2.18	118.55 ± 3.28	228.03 ± 4.36	334.16 ± 5.42	1093.30 ± 48.60
NH_3-N	4.55 ± 0.18	8.94 ± 0.41	17.14 ± 0.82	29.55 ± 1.90	88.40 ± 3.50
PO_4-P	8.35 ± 0.26	17.89 ± 0.46	34.20 ± 1.29	51.65 ± 2.33	74.20 ± 6.10
C:N:P ratio	46.83:7.01:1.0	46.70:6.77:1.0	46.55:6.69:1.0	46.89:6.47:1.0	47.02:6.28:1.0

cultured in control. *A. convolutus* grown in 40% and 60% LCRE showed significantly higher ($P < 0.05$) amounts of all the C18 and C20 PUFAs, except eicosadienoic acid (20:2n-11), than that cultured in other LCRE media and control (Table 8). In addition, *A. convolutus* grown in 60% SMRE and 10% POMED biosynthesized higher amounts of C18 and C20 PUFAs except eicosatrienoic acid (20:3n-6) and arachidonic acid (20: 4n-6) than that cultured in other SMRE and POMED media, and control (Tables 9 and 10). The micronutirents of the culture media were probably channelled into essential nutrients like unsaturated fatty acids through enzymatic activities (Laliberte and de la Noüe 1993; Johns 1994; Che *et al.* 1995; Habib *et al.* 1997 a, b).

This study showed that valuable micronutrients available in rubber and palm oil effluents may be bioaccumulated by microalgae in the form of proteins, lipids, carotene, amino acids, unsaturated fatty acids and PUFAs which can then be channelled to other organisms located higher in the food chain.

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TABLE 4
Proximate composition (g/100 g dry sample) of *Ankistrodesmus convolutus* grown in different dilutions of latex concentrate rubber effluent (LCRE), standard Malaysian rubber effluent (SMRE), digested palm oil effluent (POMED) and fertiliser. Mean(±SE) with different superscripts in each row indicate significant difference (P<0.05)

Proximate Composition	20%LCRE	40%LCRE	60%LCRE	80%LCRE	100%LCRE	Fertilizer
Moisture	5.66 ± 0.02[a]	5.60 ± 0.02[a]	5.63 ± 0.01[a]	5.62 ± 0.02 [a]	5.64 ± 0.02[a]	5.61 ± 0.01[a]
Crude protein	39.63 ± 0.82[c]	42.62 ± 1.08[a]	43.45 ± 1.18[a]	40.05 ± 0.92[c]	41.42 ± 0.98[b]	38.15 ± 0.94[d]
Crude lipid	14.05 ± 0.09[c]	15.75 ± 0.12[a]	15.72 ± 0.11[a]	15.12 ± 0.11[b]	14.32 ± 0.11[c]	10.46 ± 0.09[d]
Ash	10.45 ± 0.09[e]	12.92 ± 0.16[d]	14.29 ± 0.18[c]	17.42 ± 0.10[b]	18.68 ± 0.14[a]	8.06 ± 0.08[f]
Cardohydrate	21.98 ± 0.18[b]	18.21 ± 0.12[b]	16.33 ± 0.11[c]	16.61 ± 0.16[c]	16.24 ± 0.14[d]	21.62 ± 0.22[a]
NFE	8.20± 0.06[b]	4.86 ± 0.03[c]	3.54 ± 0.03[d]	5.14 ± 0.05[c]	3.65 ± 0.04[d]	16.06 ± 0.07[a]
Prox. Comp.	20%SMRE	40%SMRE	60%SMRE	80%SMRE	100%SMRE	N:P:K
Moisture	5.55 ± 0.02[a]	5.53 ± 0.01[a]	5.55 ± 0.01[a]	5.57 ± 0.02[a]	5.57 ± 0.02[a]	5.59 ± 0.01[a]
Crude Protein	38.68 ± 0.55[c]	39.12 ± 0.62[c]	42.02 ± 0.95[a]	40.88 ± 0.75[b]	40.52 ± 0.71[b]	38.02 ± 0.67[c]
Crude lipid	14.15 ± 0.07[b]	14.05 ± 0.07[b]	15.15 ± 0.13[a]	14.36 ± 0.11[b]	14.24 ± 0.09[b]	10.51 ± 0.06[c]
Ash	8.25 ± 0.05[e]	10.94 ± 0.07[d]	13.25 ± 0.09[c]	16.09 ± 0.09[b]	18.32 ± 0.08[a]	8.11 ± 0.07[e]
Cardohydrate	21.88 ± 0.13[a]	21.38 ± 0.12[a]	19.41 ± 0.08[b]	17.66 ± 0.10[c]	17.28 ± 0.11[c]	21.46 ± 0.13[a]
NFE	11.44 ± 0.06[b]	8.94 ± 0.06[c]	4.58 ± 0.04[d]	5.40 = 0.04[d]	4.03± 0.05[e]	16.27 ± 0.06[a]
Prox. Comp.	5%POMED	10% POMED	20% POMED		30% POMED	Fertilizer
Moisture	5.56 ± 0.01[a]	5.53 ± 0.01[a]	5.57 ± 0.01[a]		5.55 ± 0.02[a]	5.59 ±0.02[a]
Crude Protein	42.16 ± 1.06[b]	43.66 ± 1.14[a]	41.88 ± 1.23[b]		40.22 ± 1.14[c]	37.94 ± 0.89[d]
Crude lipid	16.24 ± 0.13[b]	17.68 ± 0.15[a]	14.55 ± 0.12[c]		13.39 ± 0.12[d]	10.38 ± 0.09[e]
Ash	10.66 ± 0.11[d]	13.72 ± 0.16[c]	15.96 ± 0.18[b]		18.25 ± 0.21[a]	8.15 ± 0.06[e]
Cardohydrate	20.48 ± 0.52[a]	15.59 ± 0.34[c]	17.23 ± 0.35[b]		16.90 ± 0.32[b]	21.54 ± 0.45[a]
NFE	4.85 ± 0.11[b]	3.78 ± 0.04[c]	4.77 ± 0.04[b]		4.66 ± 0.05[b]	16.36 ± 0.14[a]

TABLE 5
Amino acids (g/100g protein) of grown in *Ankistrodesmus convolutus* 20%, 40%, 60%, 80% and 100% LCRE, and fertilizer. Mean (±SE) of *essential amino acid with different superscripts in each row indicate significant differences (P<0.05)

Amino Acids	20% LCRE	40% LCRE	60% LCRE	80% LCRE	100% LCRE	N:P:K
Aspartic	13.86 ± 0.28	9.42 ± 0.17	7.46 ± 0.11	12.26 ± 0.27	11.45 ± 0.22	9.50 ± 0.17
Glutamic acid	9.55 ± 0.17	5.66 ± 0.11	5.35 ± 0.07	8.66 ± 0.21	7.25 ± 0.13	10.91 ± 0.24
Serine	5.85± 0.12	6.15 ± 0.13	6.63 ± 0.08	6.45 ± 0.16	9.25 ± 0.10	7.87 ± 0.11
Glycine	7.95 ± 0.19	7.66± 0.10	3.37 ± 0.04	7.86 ± 0.18	6.90 ± 0.09	8.55 ± 0.15
Histidine*	0.99 ± 0.02[d]	2.25 ± 0.02[b]	2.94 ± 0.03[a]	1.15 ± 0.02[d]	1.55 ± 0.02[c]	2.22 ± 0.03[b]
Arginine*	2.52 ± 0.03[c]	2.75 ± 0.03[b]	2.83 ± 0.03[b]	2.77 ± 0.03[b]	2.88 ± 0.03[b]	3.55 ± 0.04[a]
Threonine*	1.16 ± 0.02[d]	2.74 ± 0.03[b]	3.06 ± 0.04[a]	1.189 ± 0.02[c]	1.96 ± 0.02[c]	1.14 ± 0.02[e]
Alanine	14.26 ± 0.33	10.68 ± 0.22	8.17 ± 0.08	13.35 ± 0.30	12.75 ± 0.25	12.43 ± 0.19
Proline	8.15 ± 0.16	7.65 ± 0.13	6.32 ± 0.05	9.52 ± 0.22	9.15 ± 0.15	9.15 ± 0.12
Tyrosine*	2.48 ± 0.02[d]	3.15 ± 0.04[c]	5.33 ± 0.04[a]	2.42 ± 0.02[d]	2.66 ± 0.03[d]	4.01 ± 0.06[b]
Phenylalanine	1.25 ± 0.02[d]	3.45 ± 0.05[b]	4.61 ± 0.05[a]	1.35 ± 0.02[d]	1.42 ± 0.02[d]	3.14 ± 0.03[c]
Valine*	1.05 ± 0.02[e]	3.92 ± 0.04[b]	4.55 ± 0.04[a]	1.16 ± 0.02[e]	1.66 ± 0.02[d]	2.02 ± 0.02[c]
Methionine*	3.66 ± 0.06[d]	6.96 ± 0.13[b]	7.82 ± 0.12[a]	5.98 ± 0.06[c]	5.88 ± 0.10[c]	2.33 ± 0.03[e]
Cystine	2.95 ± 0.05	2.04 ± 0.04	2.93. ± 0.03	2.75 ± 0.05	2.45 ± 0.05	0.76 ± 0.02
Isoleucine*	3.05 ± 0.04[a]	2.45 ± 0.03[b]	2.47 ± 0.03[b]	2.30 ± 0.03[b]	2.35 ± 0.03[c]	2.47 ± 0.02[b]
Leucine*	2.16 ± 0.02[e]	4.75 ± 0.06[a]	4.48 ± 0.05[b]	2.05 ± 0.02[e]	2.52 ± 0.03[d]	3.35 ± 0.04[c]
Lysine*	5.88 ± 0.04[e]	8.85 ± 0.15[b]	10.15 ± 0.19[a]	6.25 ± 0.11[d]	7.58 ± 0.13[c]	4.22 ± 0.06[f]
Tryptophan*	0.75 ± 0.02[d]	1.48 ± 0.02[b]	1.66 ± 0.02[d]	0.80 ± 0.02[d]	1.05 ± 0.03[c]	0.70 ± 0.02[d]

TABLE 6

Amino acids (g/100g protein) of *Ankistrodesmus convolutus* grown in 20%, 40%, 60%, 80% and 100% SMRE, and fertilizer. Means (± SE) of *essential amino acid with different superscripts in each row indicate significant differences (P<0.05)

Amino Acids	20% SMRE	40% SMRE	60% SMRE	80% SMRE	100% SMRE	N:P:K
Aspartic	12.44 ± 0.27	12.66 ± 0.26	9.21 ± 0.19	10.55 ± 0.18	11.12 ± 0.19	9.60 ± 0.17
Glutamic acid	13.66 ± 0.32	13.75 ± 0.30	10.37 ± 0.18	11.05 ± 0.17	12.66 ± 0.21	10.85 ± 0.24
Serine	8.12 ± 0.15	6.44 ± 0.11	4.85 ± 0.04	6.12 ± 0.10	7.66 ± 0.11	7.82 ± 0.11
Glycine	6.88 ± 0.10	7.32 ± 0.13	5.78 ± 0.05	6.56 ± 0.11	5.75 ± 0.10	8.62 ± 0.15
Histidine*	1.96 ± 0.02[d]	2.05 ± 0.02[d]	2.45 ± 0.03[b]	3.15 ± 0.03[a]	2.23± 0.03[c]	2.04 ± 0.03[d]
Arginine*	2.35 ± 0.03[d]	2.38 ± 0.03[d]	4.98 ± 0.05[a]	4.57 ± 0.08[b]	2.46 ± 0.03[d]	3.46 ± 0.04[c]
Threonine*	3.01 ± 0.04[c]	3.33 ± 0.03[b]	3.97 ± 0.04[a]	3.88 ± 0.04[b]	3.25 ± 0.04[b]	1.22 ± 0.02[d]
Alanine	7.38 ± 0.15	7.45 ± 0.13	6.10 ± 0.06	7.12 ± 0.13	6.86 ± 0.13	12.45 ± 0.19
Proline	6.16 ± 0.11	5.45 ± 0.09	5.55 ± 0.05	5.16 ± 0.08	5.62 ± 0.11	9.10 ± 0.12
Tyrosine*	3.55 ± 0.04[d]	3.85 ± 0.05[c]	4.35 ± 0.04[a]	3.82 ± 0.04[c]	4.42 ± 0.07[a]	4.06 ± 0.04[b]
Phenylalanine	4.76 ± 0.05[a]	4.72 ± 0.06[a]	4.62 ± 0.04[a]	4.15 ± 0.06[b]	3.66 ± 0.04[c]	3.21 ± 0.03[d]
Valine*	4.02 ± 0.03[b]	3.98 ± 0.04[b]	4.13 ± 0.03[b]	4.52 ± 0.07[a]	4.05 ± 0.03[b]	2.06 ± 0.02[c]
Methionine*	2.55 ± 0.02[d]	2.62 ± 0.03[d]	6.22 ± 0.11[a]	4.95 ± 0.06[b]	3.75 ± 0.03[c]	2.50 ± 0.03[d]
Cystine	2.96 ± 0.03	2.96 ± 0.03	2.02 ± 0.02	3.66 ± 0.04	4.22 ± 0.04	0.85 ± 0.02
Isoleucine*	2.72 ± 0.03[d]	2.66 ± 0.02[d]	2.94 ± 0.03[c]	3.88 ± 0.03[a]	3.45 ± 0.03[b]	2.62. ± 0.02[d]
Leucine*	2.96 ±0.03[d]	3.05 ± 0.03[d]	5.36 ± 0.12[a]	4.45 ± 0.07[b]	3.36 ± 0.03[c]	3.31 ± 0.04[c]
Lysine*	3.36 ± 0.04[e]	4.16 ± 0.04[d]	9.88 ± 0.18[a]	7.18 ± 0.18[b]	5.05 ± 0.10[c]	4.29 ± 0.06[d]
Tryptophan*	1.23 ± 0.02[c]	1.32 ± 0.02[c]	1.88 ± 0.02[a]	1.72 ± 0.02[b]	1.66 ± 0.02[b]	0.68 ± 0.02[d]

TABLE 7

Amino acids (g/100 protein) of *Ankistrodesmus convolutus* grown in 5%, 10%, 20% and 30% digested palm oil effluent (POMED) and fertilizer. Means (± SE) of *essential amino acid with different superscripts in each row indicate significant differences (P<0.05)

Amino Acids	5% POMED	10% POMED	20% POMED	30% POMED	N:P:K
Aspartic	12.22 ± 0.26	10.60 ± 0.18	11.85 ± 0.25	12.85 ± 0.25	9.55 ± 0.17
Glutamic acid	10.86 ± 0.22	7.57 ± 0.14	8.66 ± 0.15	9.45 ± 0.17	10.88 ± 0.24
Serine	7.55 ± 0.13	5.24 ± 0.05	6.75 ± 0.12	7.64 ± 0.13	7.82 ± 0.11
Glycine	6.15 ± 0.11	4.13 ± 0.04	5.56 ± 0.09	5.86 ± 0.10	8.44 ± 0.15
Histidine*	2.05 ± 0.02[c]	2.98 ± 0.03[a]	2.40 ± 0.03[b]	1.75 ± 0.02[d]	2.26 ± 0.03[b]
Arginine*	2.86 ± 0.03[c]	4.97 ± 0.05[a]	3.41 ± 0.04[b]	1.66 ± 0.02[d]	3.50 ± 0.04[b]
Threonine*	3.45 ± 0.05[a]	3.50 ± 0.03[a]	3.60 ± 0.03[a]	1.24 ± 0.02b	1.12 ± 0.02[b]
Alanine	7.56 ± 0.14	5.18 ± 0.10	6.27 ± 0.13	5.88 ± 0.10	11.78 ± 0.19
Proline	8.16 ± 0.08	4.37 ± 0.08	5.86 ± 0.11	6.45 ± 0.12	9.22 ± 0.12
Tyrosine*	3.69 ± 0.05[b]	3.72 ± 0.05[b]	3.78 ± 0.04[b]	3.78 ± 0.03[b]	4.12 ± 0.06[a]
Phenylalanine	3.48 ± 0.04[b]	4.51 ± 0.06[a]	3.32 ± 0.03[b]	2.98 ± 0.02[c]	3.10 ± 0.03[c]
Valine*	2.55 ± 0.03[b]	4.63 ± 0.04[a]	2.40 ± 0.02[c]	2.35 ± 0.02[c]	2.09 ± 0.02[d]
Methionine*	4.26 ± 0.05[c]	7.22 ± 0.16[a]	5.10 ± 0.10[b]	3.05 ± 0.04[d]	2.22 ± 0.03[e]
Cystine	0.88 ± 0.02	0.63 ± 0.02	2.86 ± 0.03	4.46 ± 0.05	0.88 ± 0.02
Isoleucine*	2.15 ± 0.02[c]	3.43 ± 0.04[a]	1.15 ± 0.03[d]	1.05 ± 0.02[d]	2.36 ± 0.02[b]
Leucine*	4.25 ± 0.04[c]	6.59 ± 0.13[a]	5.46 ± 0.11[b]	3.15 ± 0.03[d]	3.28 ± 0.04[d]
Lysine*	5.09 ± 0.09[c]	7.66 ± 0.17[a]	6.16 ± 0.13[b]	5.86 ± 0.06[b]	4.16 ± 0.06[d]
Tryptophan*	1.55 ± 0.02[c]	1.86 ± 0.02[a]	1.70 ± 0.02[b]	1.72 ± 0.02[b]	0.71 ± 0.02[d]

TABLE 8
Fatty acids (g/100g lipid) of *Ankistrodesmus convolutus* grown in 20%, 40%, 60%, 80% & 100% latex concentrate rubber effluent (LCRE) and fertilizer. Means (± SE) of *unsaturated and **polyunsaturated fatty acids with different superscripts in each row indicate significant differences (P<0.05)

Fatty Acids	20% LCRE	40% LCRE	60% LCRE	80% LCRE	100% LCRE	N:P:K
Capric acid (10:0)	7.42 ± 0.07	5.72 ± 0.05	6.90 ± 0.07	7.75 ± 0.07	7.22 ± 0.07	8. 72 ± 0.09
Lauric acid (12:0)	8.57 ± 0.07	4.55 ± 0.04	4.15 ± 0.04	8.12 ± 0.04	5.28 ± 0.04	7.85 ± 0.07
Myristic acid (14:0)	9.08 ± 0.08	7.66 ± 0.06	7.12 ± 0.07	9.16 ± 0.08	9.55 ± 0.10	10.66 ± 0.10
Palmitic acid (16:0)	12.66 ± 0.11	8.38 ± 0.07	11.34 ± 0.11	12.82 ± 0.10	10.47 ± 0.09	14.05 ± 0.12
Heptadecanoic acid (17:0)	3.14 ± 0.03	3.06 ± 0.03	2.15 ± 0.02	3.25 ± 0.03	3.32 ± 0.03	3.82 ± 0.04
10-Heptadecanoic acid (17:01)*	2.04 ± 0.02[b]	1.55 ± 0.02[d]	1.24 ± 0.01[e]	1.76 ± 0.01[c]	1.47 ± 0.02[d]	2.96 ± 0.03[a]
Stearic acid (18:0)	11.27 ± 0.09	12.71 ± 0.12	10.55 ± 0.09	11.25 ± 0.11	11.82 ± 0.12	12.16 ± 0.11
Oleic acid (18:1n-9)*	5.15 ± 0.05[b]	5.62 ± 0.06[a]	5.66 ± 0.05[a]	5.25 ± 0.05[b]	5.20 ± 0.06[b]	5.05 ± 0.04[b]
Linoleic acid (18:2n-6)*	4.56 ± 0.04[c]	6.70 ± 0.06[a]	6.88 ± 0.06[a]	4.72 ± 0.04[c]	5.66 ± 0.05[b]	4.62 ± 0.08[c]
Linoleic acid (18:3n-3)**	9.47 ± 0.08[c]	13.88 ± 0.11[a]	14.06 ± 0.12[a]	9.55 ± 0.08[c]	11.44 ± 0.10[b]	9.87 ± 0.10[c]
γ-Linoleic acid (18:3n-3)**	1.25± 0.02[b]	1.58 ± 0.02[a]	1.66 ± 0.02[a]	1.22 ± 0.02[b]	1.27 ± 0.02[b]	1.13 ± 0.02[c]
Arachidic acid (20:0)	2.62 ± 0.02	4.46 ± 0.05	3.15 ± 0.03	4.18 ± 0.05	5.12 ± 0.04	2.82 ± 0.02
Eicosadienoic acid (20:2n-11)*	1.71 ± 0.02[a]	1.62 ± 0.02[a]	1.70 ± 0.02[a]	1.66 ± 0.02[a]	1.72 ± 0.03[a]	0.83 ± 0.02[b]
Eicosadienoic acid (20:3n-6)**	1.76 ± 0.03[b]	2.72 ± 0.03[a]	2.82 ± 0.03[a]	1.78 ± 0.03[b]	1.86 ± 0.03[b]	1.20 ± 0.02[c]
Arachidonic acid (20:4n-6)**	2.42 ± 0.03[c]	3.37 ± 0.03[a]	3.48 ± 0.03[a]	2.55 ± 0.03[c]	3.13 ± 0.03[c]	0.51 ± 0.02[d]
Eicosapentaenoic acid (20:5n-3)**	1.45 ± 0.02[c]	2.48 ± 0.03[a]	2.59 ± 0.03[a]	1.35 ± 0.02[c]	2.34 ± 0.03[b]	1.09 ± 0.02[d]

TABLE 9
Fatty acids (g/100g lipid) of *Ankistrodesmus convolutus* grown in 20%, 40%, 60%, 80% & 100% standard Malaysian rubber effluent (SMRE) and fertilizer. Means (± SE) of *unsaturated and **polyunsaturated fatty acids with different superscripts in each row indicate significant differences (P<0.05)

Fatty Acids	20% SMRE	40% SMRE	60% SMRE	80% SMRE	100% SMRE	N:P:K
Capric acid (10:0)	8.82 ± 0.16	7.33 ± 0.14	6.30 ± 0.15	6.76 ± 0.13	7.44 ± 0.15	8.75 ± 0.09
Lauric acid (12:0)	6.95 ± 0.07	5.77 ± 0.06	4.48 ± 0.07	5.50 ± 0.07	5.60 ± 0.08	7.80 ± 0.07
Myristic acid (14:0)	9.33 ± 0.17	8.25 ± 0.17	5.88 ± 0.16	6.96 ± 0.14	7.15 ± 0.16	10.61 ± 0.10
Palmitic acid (16:0)	12.55 ± 0.09	13.75 ± 0.10	10.58 ± 0.09	10.22 ± 0.09	11.38 ± 0.09	14.09 ± 0.12
Heptadecanoic acid (17:0)	2.03 ± 0.02	2.15 ± 0.02	1.92 ± 0.02	1.88 ± 0.02	1.69 ± 0.02	3.77 ± 0.04
10-Heptadecanoic acid (17:01)*	1.04 ± 0.02[b]	0.89 ± 0.02[c]	0.86 ± 0.02[c]	0.91 ± 0.02[c]	0.88 ± 0.02[c]	2.92 ± 0.03[a]
Stearic acid (18:0)	14.32 ± 0.12	12.55 ± 0.13	11.15 ± 0.12	12.58 ± 0.13	12.73 ± 0.15	13.13 ± 0.11
Oleic acid (18:1n-9)*	3.55 ± 0.04[d]	3.66 ± 0.04[d]	4.95 ± 0.03[a]	4.72 ± 0.04[b]	4.67 ± 0.03[b]	4.48 ± 0.04[c]
Linoleic acid (18:2n-6)*	5.34 ± 0.04[c]	5.55 ± 0.05[c]	7.75 ± 0.04[a]	6.48 ± 0.03[b]	6.62 ± 0.04[b]	5.27 ± 0.08
Linoleic acid (18:3n-3)**	9.65 ± 0.10[d]	11.66 ± 0.11[c]	15.79 ± 0.12[a]	13.49 ± 0.11[b]	1152. ± 0.11[c]	9.86 ± 0.10d
γ-Linoleic acid (18:3n-3)**	1.66 ± 0.02[b]	1.52 ± 0.02[c]	1.88 ± 0.02[c]	1.72 ± 0.02[b]	1.69 ± 0.02[b]	1.05 ± 0.03[d]
Arachidic acid (20:0)	5.25 ± 0.12	5.33 ± 0.12	3.69 ± 0.11	4.39 ± 0.11	3.45 ± 0.12	2.86 ± 0.03
Eicosadienoic acid (20:2n-11)*	0.79 ± 0.02[d]	0.88 ± 0.02[d]	2.55 ± 0.02[b]	2.12 ± 0.12[b]	1.52 ± 0.02[c]	0.89 ± 0.02[d]
Eicosadienoic acid (20:3n-6)**	1.85 ± 0.06[c]	1.92 ± 0.06[c]	3.10 ± 0.05[a]	2.99 ± 0.05[a]	2.68 ± 0.05[b]	1.27 ± 0.03[d]
Arachidonic acid (20:4n-6)**	2.26 ± 0.03[c]	2.33 ± 0.03[c]	2.77 ± 0.03[b]	2.66 ± 0.03[b]	2.94 ± 0.04[a]	0.44 ± 0.02[d]
Eicosapentaenoic acid (20:5n-3)**	1.95 ± 0.03[d]	1.95 ± 0.03[c]	2.59 ± 0.03[a]	2.48 ± 0.03[a]	2.28 ± 0.03[b]	1.02± 0.03[e]

TABLE 10
Fatty acids (g/100g lipid) of *Ankistrodesmus convolutus* grown in 5%, 10%, 20% and 30% POMED and N:P:K. Means (± SE) of *unsaturated and **polyunsaturated fatty acids with different superscripts in each row indicate significant differences ($P < 0.05$, df, 39, 5)

Fatty Acids	5% POMED	10% POMED	20% POMED	30% POMED	N:P:K
Capric acid (10:0)	5.02 ± 0.06	5.15 ± 0.08	5.99 ± 0.05	7.30 ± 0.04	8.71 ± 0.09
Lauric acid (12:0)	6.45 ± 0.11	5.95 ± 0.12	6.34 ± 0.10	7.48 ± 0.11	7.83 ± 0.07
Myristic acid (14:0)	8.91 ± 0.30	8.55 ± 0.11	7.26 ± 0.12	6.33 ± 0.10	10.64 ± 0.10
Palmitic acid (16:0)	12.33 ± 0.21	12.32 ± 0.20	14.02 ± 0.17	13.69 ± 0.19	14.05 ± 0.12
Heptadecanoic acid (17:0)	1.75 ± 0.02	1.88 ± 0.02	2.15 ± 0.03	1.55 ± 0.04	3.74 ± 0.04
10-Heptadecanoic acid (17:01)*	1.06 ± 0.02^{c}	1.25 ± 0.02^{c}	2.08 ± 0.03^{b}	2.02 ± 0.03^{b}	2.88 ± 0.03^{a}
Stearic acid (18:0)	14.92 ± 0.18	11.88 ± 0.21	13.25 ± 0.16	13.66 ± 0.11	13.90 ± 0.15
Oleic acid (18:1n-9)*	5.76 ± 0.11^{b}	6.05 ± 0.14^{a}	5.12 ± 0.08^{c}	4.32 ± 0.07^{d}	4.43 ± 0.04^{d}
Linoleic acid (18:2n-6)*	6.02 ± 0.04^{c}	8.44 ± 0.04^{a}	6.26 ± 0.05^{c}	5.40 ± 0.03^{d}	5.17 ± 0.08^{d}
Linoleic acid (18:3n-3)**	10.02 ± 0.18^{b}	15.88 ± 0.22^{a}	10.12 ± 0.14^{b}	8.44 ± 0.11^{c}	8.20 ± 0.10^{c}
γ-Linoleic acid (18:3n-3)**	2.35 ± 0.02^{a}	2.42 ± 0.02^{a}	1.75 ± 0.02^{b}	1.52 ± 0.02^{c}	1.04 ± 0.01^{d}
Arachidic acid (20:0)	3.66 ± 0.12	2.16 ± 0.11	5.32 ± 0.14	4.55 ± 0.14	1.82 ± 0.02
Eicosadienoic acid (20:2n-11)*	0.59 ± 0.03^{d}	0.65 ± 0.03^{d}	1.28 ± 0.03^{b}	1.45 ± 0.03^{a}	0.87 ± 0.02^{c}
Eicosadienoic acid (20:3n-6)**	1.45 ± 0.03^{c}	1.79 ± 0.04^{b}	1.55 ± 0.03^{c}	2.37 ± 0.03^{a}	1.22 ± 0.02^{d}
Arachidonic acid (20:4n-6)**	2.60 ± 0.03^{b}	2.80 ± 0.03^{a}	1.34 ± 0.02^{c}	1.38 ± 0.02^{c}	0.49 ± 0.02^{c}
Eicosapentaenoic acid (20:5n-3)**	1.82 ± 0.03^{a}	1.86 ± 0.04^{a}	1.64 ± 0.02^{b}	1.60 ± 0.02^{b}	1.07 ± 0.02^{c}

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