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The Effects of Different Nutrition Medium on Fat and Fatty Acid Composition of *Spirulina platensis*

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Abstract: In this study, Dry Chicken Manure (DCM) was used as the source of nitrogen in the production of *Spirulina platensis*. In the course of the experiment, the rate of the culture volume was adjusted to 10 L and the preliminary use of algae concentration was adjusted as being 500 filament mL^{-1}. The study was conducted in two different nutrition medium for 10 days with discontinuous production mode. First nutrition medium was constituted by adding the following respectively, II: group 2 mg L^{-1} urea; III: group 2 mg L^{-1} urea and 40 mg L^{-1} sodium bicarbonate; IV: group 40 mg L^{-1} sodium bicarbonate. Second nutrition medium was constituted by adding the following respectively, II: group 1 mg L^{-1} urea; III: group 1 mg L^{-1} urea and 20 mg L^{-1} sodium bicarbonate; IV: group 20 mg L^{-1} sodium bicarbonate. The 1 group of both nutrition mediums contains only chicken manure. The fat and fatty acid analyses of *S. platensis* which is obtained in the nutrition medium had been conducted. Statistically, no kind of discrepancy was noted between the two different nutrition mediums in terms of lipid levels ($p>0.05$). In the study, basic fat acids are determined as follows: capric acid (C10:0), lauric asid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), Cis-10 heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1ω9), linoleic acid (C18:2ω6), gamma linolenic acid (C18:3ω6), alfa linolenic acid (C18:3ω3).

Key words: *Spirulina platensis*, dry chicken manure, lipid, fatty acids, sodium bicarbonate

INTRODUCTION

Spirulina platensis is being produced commercially in nutrient and special nutrition forms for the last 20 years (Belay, 2002). *Spirulina* sp. is a rich cyanobacteria in terms of protein, mineral and essential fatty acids. The dry weight of Spirulina algae is consisted of 60-70% protein. Furthermore, it is also known as being one of the rare sources of Gamma Linoleic Acid (GLA). Spirulina is often used in the nutrient and nourishment studies due to it's ability of producing pigments, fatty acids, carbohydrates, proteins and many other nutrient compounds. *S. platensis*, finds it's place in the market as being a nutraceutical and a pharmaceutical additive.

Microalgae are in the need of basic elements such as Carbon (C), Nitrogen (N) and Phosphorus (P) in order to conduct the organic substance synthesis. In cultures, the most important inorganic 'N' sources that are able to be used by the cells are as follows; NO_3 nitrate (NO_3-NN), NH_4 nitrate (NH_4-N) and urea nitrate ($(NH_2)_2CO$-N). Due to all the 'N' sources are considered within the structure of amino acids and thereby proteins which are being the main constituents of a cell, they have vital values. Furthermore, 'N' sources are also known as having enormous effects on fatty acids. As being the main constituent of nitrate enzymes and proteins, it is needed in the synthesis of fatty acids. Nitrate sources and concentrations effect the growth and biochemical composition and cause variations particularly on fatty acid values (Shifrin and Chisholm, 1981; Sukenik *et al*., 1989).

MATERIAL AND METHODS

S. platensis which is used in the study was produced in the "Plankton Laboratory of Fisheries Faculty of Mersin University". As the culture medium Spirulina liquid medium was used by having been modified. In the modified Spirulina medium $NaNO_3$ was used.

For vaccination culture, first of all, the cells of Spirulina are filtered with a plankton cloth in 30 μm pores. It was washed 3 times with 0.8% NaCl solution in order to purify from sodium nitrate. Afterwards, the plantation of Spirulina cells into the modified Spirulina medium was applied. Initial pH-value was stabilized at 10 and the temperature at 34±1°C and the lightening process had been applied for 24 h. When the culture reached up to a maximum desired density, the plantation into manure syrup which is prepared by filtering was conducted.

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Table 1: Nutritional medium used in the experiment

Groups	Nutrition medium	Experiments
I. Nutrition medium		
I	DCM	Filtered and auto-claved tap water every 24 h
II	DCM+Urea	Urea every 24 h (2.0 mg L^{-1})
III	DCM+Urea+$NaHCO_3$	Urea every 24 h (2.0 mg L^{-1}) and (40 mg L^{-1}) sodium bicarbonate ($NaHCO_3$)
IV	DCM+$NaHCO_3$	40 mg L^{-1} sodium bicarbonate was added every 24 h
II. Nutrition medium		
I	DCM	Filtered and auto-claved tap water every 24 h
II	DCM+Urea	Urea every 24 h (1.0 mg L^{-1})
III	DCM+Urea+$NaHCO_3$	Urea every 24 h (1.0 mg L^{-1}) and (20 mg L^{-1}) sodium bicarbonate
IV	DCM+$NaHCO_3$	20 mg L^{-1} sodium was added every 24 h

DCM: Dry Chicken Manure

In the trial culture medium 200 g of dry chicken manure was used. Dry chicken manure was powdered by food grinding machine. The powdered dried chicken manure was sieved through and was kept waiting by being aerated in the tap water that is filtered by a filtration system, in 10 L plastic cups for 7 days. Furthermore, sodium metabisulfite (5 mg L^{-1}) is added into the medium in order to prevent the microbial transmission 24 h after the commence of the trial 8.5 g L^{-1} of sodium bicarbonate was also added.

The trial was conducted in 10 L of glass cups in a discontinuous mode. Every culture was aerated by a central aeration system. The trial had been conducted for 10 days.

The nutrition mediums and quantities that are used in the experiment are given in (Table 1). The experiments were conducted in two different nutrition medium on 4×3 experimental designs, in 8 groups, each three times repeatedly.

Vaccination: The Spirulina cells (in 500/filament/mL density) that are purified from nitrate were vaccinated into the manured water. Manured culture medium was prepared by the vaccination of the Sprirulina cells that was purified from the 5.5 L manured and 4.5 L nitrate.

Obtaining of Spirulina platensis harvest: In the end of the experiment *S. platensis* products obtained from the 1st and 2nd nutrition medium, each group on their own are filtered through a plankton cloth with a 30 μm pores. The products that are obtained had been kept in the laboratory medium for 1 day and then the drying process was applied at 50°C temperature for three days in the incubator. The dried products before analyzing process had been preserved in the refrigerator.

Fat and fatty acids analysis: Total lipid analysis Bligh and Dyer (1959) was conducted according to the extraction method. Methyl esters of fatty acids are prepared according to the method which was developed by modifying by Ichihara *et al.* (1996). Adding 2 mL n-heptane (Merck 1.04365.2500) and 4 mL 2 M methanolic KOH (Merck 1.09112.1000) into the volumetric flasks which contain lipid was shaken until the whole lipid penetrates into the dissolvent. Afterwards, the lipid solution was transferred from the volumetric flasks into the lidded centrifuge tubes for centrifuge process. The samples which are centrifuged in frigorific centrifuge at 400 rpm for ten minutes were transferred into the dilution tubes by removing the supernatant with Pasteur pipettes. The samples in the form to contain 20-25 μg lipid in their mL are readied for injection by being diluted with heptane. Finally, the samples which were transferred into the vials placed into GC and their injections were conducted. Fatty acid compositions were analyzed by the help of flame ionization detector and a silica capillary SGE column (30 m X 0.32 mm ID X 0.25 μm BP20 0.25 UM, USA) Clarus 500 (Perkin Elmer, USA) that contains gas chromatography with auto sampler. Injector and FID detector temperatures are adjusted to 220 and 280°C, respectively. The furnace temperature for the first 5 min were kept at 140°C degrees and afterwards brought to 200°C, raising 4°C at a minute, from 200-220°C, raising 1°C at a minute. The amount of sample is being 1 μL and the control of the carrier gas kept at 16 ps. The injection application was conducted at the ratio of 1:50. Fatty acids compositions were defined by retention time of FAME mixture that is consisted of 37 standard compounds.

Statistical analysis: The statistical analysis were made using SPSS 16.0 Software package and the comparisons were carried out with t-test and one-way ANOVA Duncan Method.

RESULTS AND DISCUSSION

In this study, chicken manure was used as the source of nitrate for *S. platensis* culture. In one study that was conducted, it is stated that chicken manure meets the requirements for the necessary source of nourisment for *S. platensis*. In another study, it is stated that the manured water that is obtained from the digested pig waste is a low cost alternative for big scale Spirulina production (Olguin *et al.*, 1997). Also in another study, it is reported that the use of urea as the nitrate source

in *S. platensis* cultures is one of the important factors which affects the biochemical composition and the growth in alga cultures (Stanca and Popovici, 1996; Xu *et al*., 2001).

The commercial production productivity of *Spirulina* sp. concentrates on growth conditions of optimum biomass production. In general, the production of *Spirulina* sp. is being conducted regardless of it's chemical composition. However, with the manipulation of the growth conditions of *Spirulina* sp., compounds such as multiple unsaturated fatty acids can be obtained in high concentrations. Whilst the growth conditions effect the development of *Spirulina* sp., the changes occurring in it's metabolism also alters it's composition of biomass (Cornet *et al*., 1992).

In terms of the lipid levels between the two different nutrition mediums, there are no statistical discrepancies noted ($p>0.05$). There are differences noted between the I and II groups and III and IV groups of the I. nutrition medium ($p<0.05$). There are also statistical differences between the I, II, III and IV groups of the II. nutrition medium ($p<0.05$). The lipid levels of *S. platensis* produced in the I. and II. groups of both nutrition mediums is found to be higher than those in the III and IV groups (Table 2). The reason for this is stated to be the result of sodium bicarbonate addition to the III and IV groups. Vonshak *et al*. (1982) stated that this eventuates due to the partial pressure of CO_2 of high biomass values being so in high temperatures and as result of the increasing bicarbonate ratios and the acceleration of photosynthesis. The results that the researchers had notified, supports our findings.

Basic fatty acids of *S. platensis* produced in two different nutrition medium with DCM are determined as being; capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), Cis-10 heptadecanoic acid (C17:1), stearic acid (C18:0), oleic ascid (C18:1ω9), linoleic acid (C18:2ω6), gamma linolenic acid (C18:3ω6), alfa linolenic acid (C18:3ω3) (Table 3). By Muller *et al*. (1994), the most commend fatty acids in the fatty acid profile of *Spirulina* sp. are

Table 2: Variations in lipid levels of *S. platensis* in different nutritional medium (%)

Lipid	I group ($\bar{X}\pm S_{\bar{x}}$)	II group ($\bar{X}\pm S_{\bar{x}}$)	III group ($\bar{X}\pm S_{\bar{x}}$)	IV group ($\bar{X}\pm S_{\bar{x}}$)
I. Nutrition medium	$4.82\pm0.22^{x,b}$	$4.77\pm0.44^{x,b}$	$3.80\pm0.78^{x,a}$	$2.63\pm0.60^{x,a}$
II. Nutrition medium	$4.96\pm0.12^{x,d}$	$4.62\pm0.21^{x,c}$	$3.84\pm0.03^{x,b}$	$2.59\pm0.52^{x,a}$

Different lowercase letters in a column (x, y) and in a row (a, b, c, d) indicate significant differences ($p<0,05$). $\bar{X}\pm S_{\bar{x}}$: Mean±Standard error

Table 3: Variations in fatty acid composition of *S. platensis* in different nutritional medium (%)

Fatty acids	I group ($\bar{X}\pm S_{\bar{x}}$)	II group ($\bar{X}\pm S_{\bar{x}}$)	III group ($\bar{X}\pm S_{\bar{x}}$)	IV group ($\bar{X}\pm S_{\bar{x}}$)	NM
C10:0	$1.42\pm0.48^{x,a}$	$2.46\pm1.17^{y,a}$	$2.16\pm0.22^{y,a}$	$1.97\pm1.21^{x,a}$	I.
	$0.93\pm0.10^{x,b}$	$0.75\pm0.23^{x,b}$	$0.34\pm0.06^{x,a}$	$2.93\pm0.47^{x,c}$	II.
C12:0	$1.33\pm0.36^{x,a}$	$1.49\pm0.08^{y,a}$	$1.98\pm0.17^{y,b}$	$2.13\pm0.69^{y,b}$	I.
	$1.09\pm0.16^{x,b}$	$0.59\pm0.19^{x,a}$	$0.62\pm0.15^{x,a}$	$1.04\pm0.11^{x,b}$	II.
C14:0	$2.80\pm0.77^{y,b}$	$2.49\pm0.31^{y,b}$	$3.12\pm0.55^{y,b}$	$1.63\pm0.10^{x,a}$	I.
	$1.36\pm0.08^{x,c}$	$0.62\pm0.12^{x,a}$	$0.98\pm0.11^{x,b}$	$1.43\pm0.32^{x,c}$	II.
C14:1	$5.01\pm0.07^{y,c}$	$2.05\pm0.57^{x,a}$	$3.11\pm0.93^{y,ab}$	$4.11\pm0.57^{y,b}$	I.
	$2.52\pm0.03^{x,b}$	$1.79\pm0.31^{x,a}$	$1.71\pm0.36^{x,a}$	$2.43\pm0.13^{x,b}$	II.
C16:0	$12.06\pm1.75^{x,a}$	$16.60\pm1.45^{x,b}$	$12.75\pm1.20^{x,a}$	$11.79\pm1.62^{x,a}$	I.
	$16.53\pm0.66^{y,a}$	$20.47\pm1.75^{y,b}$	$25.35\pm0.63^{y,c}$	$25.28\pm0.33^{y,c}$	II.
C16:1	$19.88\pm0.96^{y,b}$	$17.23\pm0.45^{x,a}$	$21.93\pm0.53^{y,c}$	$24.42\pm0.26^{y,d}$	I.
	$14.56\pm0.63^{x,a}$	$15.20\pm2.15^{x,a}$	$14.21\pm1.05^{x,a}$	$14.39\pm1.63^{x,a}$	II.
C17:0	$0.48\pm0.10^{y,b}$	$0.11\pm0.02^{x,a}$	$0.48\pm0.04^{y,b}$	$0.57\pm0.09^{x,b}$	I.
	$0.15\pm0.00^{x,a}$	$0.49\pm0.07^{y,b}$	$0.15\pm0.04^{x,a}$	$0.53\pm0.04^{x,b}$	II.
C17:1	$0.56\pm0.06^{x,a}$	$1.18\pm0.18^{y,b}$	$1.17\pm0.18^{y,b}$	$1.01\pm0.13^{y,b}$	I.
	$0.53\pm0.04^{x,b}$	$0.78\pm0.01^{x,c}$	$0.26\pm0.04^{x,a}$	$0.25\pm0.07^{x,a}$	II.
C18:0	$3.93\pm0.17^{y,b}$	$4.77\pm0.39^{y,c}$	$1.99\pm0.16^{x,a}$	$3.09\pm0.41^{x,b}$	I.
	$2.72\pm0.13^{x,b}$	$1.43\pm0.34^{x,a}$	$1.73\pm0.13^{x,a}$	$2.67\pm0.05^{x,b}$	II.
C18:1ω9	$8.16\pm1.21^{y,c}$	$6.15\pm0.07^{y,b}$	$4.05\pm0.36^{x,a}$	$5.37\pm0.65^{x,b}$	I.
	$3.79\pm0.16^{x,a}$	$4.77\pm0.19^{x,b}$	$3.81\pm0.05^{x,a}$	$5.05\pm0.52^{x,b}$	II.
C18:2ω6	$7.58\pm0.54^{y,c}$	$12.88\pm2.16^{y,d}$	$5.94\pm0.07^{y,a}$	$6.74\pm0.06^{y,b}$	I.
	$3.04\pm0.17^{x,a}$	$4.79\pm0.08^{x,c}$	$3.89\pm0.28^{x,b}$	$5.45\pm0.25^{x,d}$	II.
C18:3ω6	$3.50\pm0.54^{x,c}$	$2.55\pm0.18^{x,b}$	$9.45\pm0.22^{y,d}$	$1.92\pm0.05^{x,a}$	I.
	$6.15\pm0.23^{y,a}$	$9.35\pm0.18^{y,c}$	$7.99\pm0.83^{x,b}$	$6.05\pm0.10^{y,a}$	II.
C18:3ω3	$2.37\pm0.03^{x,a}$	$2.30\pm0.04^{x,a}$	$5.95\pm0.66^{x,b}$	$2.62\pm0.50^{x,a}$	I.
	$3.82\pm0.15^{y,a}$	$8.36\pm0.25^{y,d}$	$6.97\pm0.81^{x,c}$	$5.11\pm0.16^{y,b}$	II.
∑SFA	$22.00\pm4.23^{x,a}$	$27.90\pm3.42^{x,b}$	$22.46\pm2.94^{x,a}$	$21.16\pm4.42^{x,a}$	I.
	$22.78\pm1.14^{x,a}$	$24.36\pm3.20^{x,a}$	$29.15\pm1.21^{y,b}$	$33.86\pm1.51^{y,b}$	II.
∑MUFA	$33.60\pm2.30^{y,bc}$	$26.60\pm2.87^{x,a}$	$30.25\pm2.01^{y,b}$	$34.90\pm2.62^{y,c}$	I.
	$21.40\pm0.86^{x,ab}$	$22.55\pm2.67^{x,b}$	$19.97\pm1.50^{x,a}$	$22.11\pm2.36^{x,ab}$	II.
∑PUFA	$13.45\pm1.10^{x,b}$	$17.73\pm1.38^{x,c}$	$21.34\pm1.96^{y,d}$	$11.27\pm0.62^{x,a}$	I.
	$13.00\pm0.55^{x,a}$	$22.49\pm0.51^{y,d}$	$18.84\pm0.92^{x,c}$	$16.61\pm1.00^{y,b}$	II.

Different lowercase letters in a column (x, y) and in a row (a, b, c, d) indicate Significant differences ($p<0.05$). $\bar{X}\pm S_{\bar{x}}$: Mean±Standard Error; NM: Nutrition Medium

stated to be palmitic acid (C16:0), gamma linolenic acid (C18:3n6) and linoleic acid (C18:2n6). The notified results in both studies match up with our findings that we obtained.

Van Rijn and Shilo (1986) stated in their studies they had conducted in 1986 that the changes of temperature and nitrate ingredients caused significant impacts on palmitoleic acid (C16:1) concentrations. Researchers also stated that increasing of the sodium nitrate ingredients causes negative effects on linoleic and palmitoleic acid concentration. In this study, decrease on the level of palmitoleic acid was noted in conjunction with the increase of 'N' in the nutrition medium (Table 3). The results that Rijn and Shilo had notified support our findings. Spirulina containing gamma-linoleic acid (C18:3, ω6, GLA) is one of the most attractive fatty acid for the researchers (Sarada *et al*., 1999).

GLA is commonly examined due to it's ability to decrease cholesterol levels in blood (Yamamoto *et al*., 1988). *Spirulina* sp. has been examined as being the potential source of GLA (Alonso and Marato, 2000; Quoc *et al*., 1994; Cohen *et al*., 1987, 1993). Spirulina, to increase the ingredient of GLA, conditions such as growing conditions (Quoc *et al*., 1994), low growth tempratures (Tedesco and Duerr, 1989; Cohen *et al*., 1987), nitrate source (Tedesco and Duerr, 1989; Piorreck *et al*., 1984), culture age and lightening (Olguin *et al*., 2001; Quoc *et al*., 1994; Cohen *et al*., 1987). Tedesco and Duerr (1989) and Piorreck *et al*. (1984) in the study they had conducted in 1984 stated that in addition to the chicken manure use as being the source of nitrate, the reinforcement urea and sodium causes an increase in the bicarbonate gamma linoleic acid levels. In our study, similarly in addition to chicken manure, the addition of urea and sodium bicarbonate causing an increase in the GLA and α-linolenic acid levels of Spirulina was detected (Table 3).

Koru and Cirik in a study they had conducted, they stated the levels of myristic acid, palmitic acid, stearic acid, oleic acid, gamma linolenic acid, as being in the following order; 0.78, 49.8, 1.59, 6.0 and 16.91%. In our study, the level myristic acid was found to be between 0.62-3.12%. The level that the researches stated is in parallel with the level detected in this study. In this study, the level of palmitic acid is found to be between 11.79-25.35%. The range that is obtained in our study is found to be lower than that was informed by the researches. In this study, the stearic acid and oleic acid levels are noted to be between 1.43-4.77 and 3.79-8.16%. The levels which the researchers informed for both fatty acids are in between the range that was noted in this study. The highest level of GLA was noted to be 9.45% in our study. This level is lower than that the researches had informed. By Koru and Cirik, the levels of saturated and unsaturated fatty acids are stated as being 52.17 and 47.73%. In our study, the saturated and unsaturated fatty acid levels are detected as being 33.86 and 51.0%. The levels that the researches informed support our findings.

With this study fat and fatty acids compositions of Spirulina produced in dry chicken manure were determined. As result of the findings that were obtained, linoleic acid, GLA and alfa linolenic acid levels are found to be higher than the other fatty acids levels. It is concluded that these fatty acids being important in the use of chicken manure in large scale *S. platensis* production and in human and animal nutrition can be obtained economically.

CONCLUSION

In this study, it is aimed to find out fat and fatty acids compositions of *S. platensis* which is produced in "discontinuous production mode" in the culture medium which are composed by adding urea and sodium bicarbonate into the dry chicken manure.

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