EFFECT OF *SPIRULINA* (*ARTHROSPIRA PLATENSIS*) ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF FRIESIAN COWS

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Abstract

Fifteen multiparous Friesian cows with live body weight (LBW) of 510-532 kg and 2-4 parities were used at 45 days pre-partum and continues until 120 days post-partum and divided into three similar groups (5 in each group) for study the effect of *Spirulina* additive on their productive and reproductive performance. Group 1 was unsupplemented which was served as control (G_1), or supplemented with *Spirulina* and their media at the levels of 1 ml (low level) or 2 ml (high level) / kg LBW in drinking water for tested treatments G_2 and G_3, respectively. *Spirulina* was grown in a modified Zarrouk٫s medium for 21 days. Results showed that digestibility of all nutrients and feeding values of G3 were significant higher ($P<0.05$) than those of either of G1 or G2, and also all values of these items were significant higher than those of G1. Total DMI was insignificant increased with G2 and significant increased with G3 compared with that of G1. Ruminal pH values did not significant affected by treatments, while TVFA's was increased significantly with increasing *Spirulina* supplement in dietary treatments and NH_3-N values had an inverse trend among treatments. Most blood biochemical and haematological parameters were markedly increased with increasing the algae level in drinking water of cows. Live body weight and body condition score decreased gradually until reach 90, 75 and 60 days and increased thereafter for G_1, G_2 and $G_{3,}$ respectively. Group 3 recorded significantly ($P<0.05$) the highest daily yield of actual milk and 4%-FCM, colostrum and milk composition, feed and economic efficiencies and the lowest electrical conductivity in milk. Concerning reproductive traits, cows in G_3 recorded significantly ($P<0.05$) the short periods from parturition until the first estrus and insemination, as well as days open, the highest conception rate and the lowest number of service per conception. In conclusion, cows supplemented with *Spirulina* and their media at the level of 2 ml/kg LBW in drinking water showed the best results concerning productive performance and body condition score as well as post-partum reproductive traits.

Keywords: *Spirulina* additives, cows, productive performance, body condition score and reproductive traits.

INTRODUCTION

The profitability of dairy farming primarily depends on the productivity of the cow herd, which, in turn, much longer depends on the conditions of nutrition. The positive impact is the result of genetic characteristics, improved nutrition and keeping on optimal conditions of the cattle, and the experience of dairy farmers. While productivity is growing, the nutritional needs also change depending on the animal's age, physiological state and lactation period (Ulbricht *et al*., 2004). In addition, most nutritional substances in the ration have to be in the proper proportion; as a result, balancing rations for high productivity cows are not an easy task. Under prevailing local conditions particularly in summer season where most rations of cows are inclusion a relatively high proportion of poor quality roughage (straw), extremely lack of energy and protein, which forced cows to compensate their nutritional needs from the body reserves, resulting the decrease body weight and induce some metabolic disorders. If the body condition at calving matches the standards and in the first weeks after calving the rations are properly balanced, it would be possible to avoid the negative effects on milk production (Dechow *et al*., 2002). *Arthrospira platensis* (*Spirulina*) organic materials consisted of proteins 60-70%, carbohydrates 10-20%, fat 5% and fiber 2% (Hendrickson, 1989). According to the findings of Paulauskas and Kulpys (2007) who compared with proteins of other raw materials and found that proteins of *Arthrospira platensis* (*Spirulina*) have more valuable amino acids as well as biologically active substances such as vitamins, minerals, polysaccharides, which are able to form an extremely beneficial compound with metals, etc. Among biologically active compounds, isoprenoids that are importantly and positively influencing the activity of various enzymes, as well as the synthesis of nucleic acids and photosynthesis. Antioxidant characteristics of cyanobacteria are based on its contents of phytohormones and enzymes. Because of its biologically active substances, unique chemical composition, valuable proteins, correct proportion of amino acids, and amounts of vitamins and minerals, the biomass of *Spirulina* algae can be successfully used in animal nutrition (Wallace, 2000). Cow's milk productivity increased by 7.6 % after receiving feed additive of *Spirulina* (Simkus *et al*., 2008). Recently, started to grow cyanobacteria *Arthrospira platensis* (*Spirulina*) artificially for preparation this effective additive for animal nutrition. The combined fodder with *Spirulina* additive in feeding cows at early lactation period had a positive influence as increasing milk productivity by 21%. That also increased the yield of milk fat, protein and lactose. *Spirulina* additive was positively influenced the induction of cow estrus and body condition (8.5-11%). Fodder additive cyanobacterium (*Spirulina platensis*) used in the early lactation period, was economically effective, compared to the cows feeding without this additive (Kulpys *et al.*, 2009).

The objective of this study was to investigate the effect of adding fresh *Spirulina* algae with their media in drinking water on feed intake, digestibility, rumen fermentation activity, blood biochemical and haematological parameters, milk yield and composition, electrical conductivity, feed conversion, economic efficiency, body weight change during parturition and post-partum reproductive traits of Friesian cows.

MATERIALS AND METHODS

The present study was carried out at Sakha Animal Production Research Station, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt.

***Spirulina* cultivation**

Spirulina algae was cultivated in Cyanobacteria Research Lab., Microbiology Dept., Sakha Agricultural Research Station, Soils, water, and Environment Research Institute, Kafr El-Sheikh, Egypt. *Spirulina* algae was grown in a modified Zarrouk's medium (Ravelonandro *et al.*, 2008). The composition of the medium was per liter of distilled water: 10 g $NaHCO_3$, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$, 1.0g K_2SO_4, 1.0 g NaCl, 0.20 g $MgSO_4 \cdot 7H2O$, 0.01 g $FeSO4 \cdot 7H_{2O}$, 0.08 g EDTA and 0.04 g $CaCl_2$ and added 1.0 mL. microelements per liter of medium, 2.86 g H_3Bo_3, 1.81 g Mn $Cl_2.4H_2o$, 0.222g $ZnSo_4.7H_2o$, 0.079 g $CuSo_4.5H_2o$, 0.39 g $NaMoO_4$ and 0.0494g $Co(No_3)_2.6H_2o$. In which a 100 ml of the medium was transported to Erlenmeyer glass flask and autoclaved for 20 min. at 121 ^oC. The flask was inoculated with 10 ml culture and incubated for 7 days, then transported to 1000 ml of media and incubated for 7days; finally, the growth was transported to polyethylene container 20 L (square in shape, a white and transparent) and incubated for 21 days for *Spirulina* mass culture. All flask and containers were grown under controlled laboratory conditions in the chamber at temperature 30±2^{o}C under continuous light, produced by fluorescent white bulbs of 120 cm length and fluorescence illumination (5500 - 6500 lux). The body of the container was cleaned externally with ethanol 70% and sterilize distilled water several times. The air was obtained into the container by the air pump (Model AP -005, Pressure 0.02 × 2 m pa, Power SW and L. Max 2.5) to circulate of the culture media which have two holes each connected with a plastic tube (0.5 mm diameter and 120 cm length). At the end of the plastic tube found air distributor to distribute the air regularly in the container.

Animals and experimental groups

Fifteen multiparous Friesian cows with live body weight (LBW) of 510-532 kg and 2-4 parities were used at 45 days pre-partum and continues until 120 days post-partum and divided into three similar groups (5 in each group). Cows were fed a basal ration contained (on DM basis) 50% concentrate feed mixture (CFM), 15% fresh berseem (FB), 15% corn silage (CS) and 20% rice straw (RS) without any supplement in G_1 which was served as control or supplemented with *Spirulina* algae and their media at the levels of 1 ml (low level) or 2 ml (high level) / kg LBW in drinking water for G_2 and G_3, respectively. Chemical composition of feedstuffs and calculated composition of a basal ration are presented in Table (1). Concentrate feed mixture consisted of 32% undecorticated cotton seed cake, 24% wheat bran, 22% yellow corn, 12% rice bran, 5% linseed cake, 3% molasses, 1% limestone and 1% common salt.

Table 1. Chemical composition of feedstuffs and basal ration.

Item	DM %	Composition of DM %					
		OM	CP	CF	EE	NFE	Ash
CFM	90.64	91.25	16.12	9.73	2.85	62.55	8.75
FB	15.43	88.86	15.94	25.17	2.29	45.46	11.14
CS	32.35	93.90	8.16	23.25	2.48	60.01	6.10
RS	89.18	82.78	2.38	35.61	1.32	43.47	17.22
Basal ration	45.21	89.60	12.15	19.25	2.40	55.80	10.40

Management procedure

Cows were housed under sheds in semi-open backyards and were fed their rations to cover their recommended requirements according to NRC (2001). Concentrate feed mixture was offered in two equal parts daily at 8 a.m. and 4 p.m., fresh berseem, corn silage and rice straw were offered once daily at 10 a.m., 12 med-day and 3 p.m., respectively. Fresh *Spirulina* with their media was added in drinking water at morning every day. Animals were free for watering all the day round.

Digestibility trials

Digestibility trials were carried out at the end of the experiment using 3 cows from each group to determine the digestibility coefficients and feeding values of the experimental rations using acid insoluble ash (AIA) as a natural marker (Van Keulen and Young, 1977). Feces samples were taken from the rectum of each cow twice daily at 12 h intervals during the collection period. Samples of feedstuffs were taken at the beginning, middle and end of the collection period. Representative samples of feedstuffs and feces were chemically analyzed according to the methods of AOAC (2012). Digestibility coefficients were calculated from the equations:-

DM digestibility % = $100 - \left[100 \text{ x } \frac{\text{AIA\% in feed}}{\text{AIA\% in feces}}\right]$

Nutrient digestibility % = $100 - \left[100 \text{ x } \frac{\text{AIA\% in feed}}{\text{AIA\% in feces}}\right] \text{x} \left[\frac{\text{Nutrient \% in feces}}{\text{Nutrient \% in feed}}\right]$

Rumen liquor samples:

Rumen liquor samples were taken from animals at the same time of digestibility trial using stomach tube attached to a vacuum pump at 4 hours post feeding. Rumen liquor samples were strained through a double layer of cheese cloth and rumen pH was measured immediately after collection using a digital pH meter (Hanna Instruments pH). Rumen liquor was preserved with a few drops of saturated mercuric chloride and frozen in labelled poly propylene bottles for estimation of total volatile fatty acids (TVFA's) and ammonia nitrogen (NH_3-N). The TVFA's concentration was determined by a steam distillation method as described by Warner (1964) and NH_3-N concentration was determined using magnesium oxide (AOAC, 2012).

Blood samples

Blood samples were taken from the jugular vein of each cow by clean sterile needle in the heparinized clean dry plastic tube after 4 hours from the morning feeding in two parts. The first part was centrifuged at 4000 rpm for 15 min to separate plasma and stored at -20 ^{o}C. Total protein, albumin, globulin (total protein - albumin), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically by spectrophotometer (Spectronic 21D, USA) using commercial kits produced by Diagnostic System Laboratories, Inc., USA. The second part was used for determined hematological parameters. Haematological analysis was performed by Medonic Vet. according to Drew *et al.* (2004). Hematology Analyzer (Medonic CA 620, Sweden) directly within 1-2 hrs after samples collection. Haematological variables measured were red blood cell count (RBC's), haemoglobin concentration (HGB), red blood cell distribution width (RDW), haematocrit (HCT), main corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Other parameters included the platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and procalcitonin (PCT). Leucocyte variables measured were white blood cell count

(WBC's) and differential white cell count, lymphocytes (LY), monocytes (MO) and granulocytes (GR).

Milk yield and samples

Cows were mechanically milked at 6 a.m. and 5 p.m. Morning and evening milk yields were recorded every day for each cow and also 4% FCM for each cow was calculated from daily milk yield and the percentage of fat in milk. Milk samples from the consecutive evening and morning milkings were taken from each cow every two weeks and mixed in proportion to milk yield. Composite milk samples were analyzed for fat, protein, lactose, solids not fat (SNF), and total solids (TS) by Milko-Scan (model 133B), and ash by difference. Electrical conductivity was measured in milk for each quarter during every milking using Dramtnski, Electronic in agriculture, Mastitis detection, Italy.

Feed conversion ratio

Feed conversion ratio was determined as the amounts of DM, TDN and DCP required for producing 1 kg 4% FCM.

Economic efficiency

Economic efficiency was calculated as the ratio between the price of produced 4% FCM and the cost of feed consumed. The prices in Egyptian pound (LE) per ton were 2750 L.E. for concentrate feed mixture, 320 L.E. for fresh berseem, 350 L.E. for corn silage, 250 L.E. for rice straw, 2 L.E./ liter fresh *Spirulina* with their media and 3.35 L.E. / kg 4% FCM produced during year 2015-2016.

Reproductive parameters

Reproductive parameters as the periods from calving to first estrus and first insemination, days open, number of service per conception and conception rate were recorded for each cow.

Statistical analysis

The data were analyzed using general linear models procedure adapted by IBM SPSS Statistics (2014) for user's guide with one-way ANOVA. Significant differences in the mean values among dietary treatments were analyzed by Duncan's tests within SPSS program set at the level of significance $P<0.05$.

RESULTS AND DISCUSSION

***Spirulina* (*Arthrospira platensis*)**

The filament count of *Spirulina* (*Arthrospira platensis)* grown in a modified Zarrouk's medium for 21 days was 21 x 10^6 cfu/ml.

Digestibility and feeding values

Nutrient digestibility coefficients and feeding values for different dietary treatments are shown in Table (2). The addition of *Spirulina* (1 and 2 ml/kg LBW) resulted in a significant (P<0.05) higher in the digestibility coefficient of DM, OM, CP, CF, EE and NFE and subsequently TDN and DCP values than control (G1). Also, significant differences between the two levels of *Spirulina* additive were found respective all nutrient digestibilities and feeding values, with the highest values were associated with the higher level (G3). The better digestibility for cows with *Spirulina* additive may be a subsequence of the high nutrient density of *Spirulina* as well as stimulation of the secretion of extracellular enzymes by the gut microflora. Total tract digestibility in pig receiving microalgae (*Spirulina* and *Chlorella*) was greater for gross energy (P<0.05), and tended to be greater for dry matter, organic matter and NDF (P<0.10) compared with standard diet with no supplementation (Tovar-Ramírez *et al.*, 2002).

Feed intake

Average daily feed intake for the different groups are presented in Table (3). There were significant differences (P<0.05) in daily feed intake among the different groups, where G3 recorded the highest feed intake followed by G2, while the control group (G1) had the lowest feed intake (P<0.05). The intake of total DM, TDN, CP and DCP increased by 1.76, 4.64, 1.49 and 5.34% for G2 and 3.63, 9.67, 3.48 and 10.69% for G3 compared with G1, respectively. The intake of TDN and DCP were more affected by dietary treatments than the intake of DM and CP and this response may be a reflection of the increase in the values of TDN and DCP as shown in Table (2). El-Sabagh *et al.* (2014) found that *Spirulina platensis* supplementation in fattening lambs diets improved feed intake, compared to the control group (P<0.05).

Table 2. Nutrient digestibility coefficients and feeding values for different dietary treatments.

Item	Experimental treatments			SEM
	G1	G2	G3	
Digestibility coefficients %				
DM	64.06^{c}	66.15^{b}	68.46^{a}	0.52
OM	65.14^{c}	67.17^{b}	69.40^{a}	0.50
CP	65.50^{c}	67.51^{b}	69.72^{a}	0.49
CF	62.26^{c}	64.46^{b}	66.88^{a}	0.54
EE	73.04^{c}	74.62^{b}	76.34^{a}	0.39
NFE	69.45^{c}	71.23^{b}	73.19^{a}	0.44
Feeding values %				
TDN	62.64^{c}	64.39^{b}	66.31^{a}	0.43
DCP	7.96^{c}	8.20^{b}	8.47^{a}	0.06

a, b, c: Values in the same row with different superscripts differ significantly (P<0.05).

Table 3. Average daily feed intake (kg/head) for different dietary treatments.

Item	Experimental treatments			SEM
	G1	G2	G3	
CFM*	9.11^{b}	9.27^{ab}	9.44^{a}	0.05
FB*	16.05^{b}	16.33^{ab}	16.63^{a}	0.09
CS*	7.65^{b}	7.79^{ab}	7.93^{a}	0.04
RS*	3.70^{b}	3.77^{ab}	3.84^{a}	0.02
Total DM	16.51^{b}	16.80^{ab}	17.11^{a}	0.09
TDN	10.34^{c}	10.82^{b}	11.34^{a}	0.12
CP	2.01^{b}	2.04^{ab}	2.08^{a}	0.01
DCP	1.31^{c}	1.38^{b}	1.45^{a}	0.02

* as fed.

a, b, c: Values in the same row with different superscripts differ significantly (P<0.05).

Rumen fermentation activities

Data of rumen fermentation activities are presented in Table (4). Ruminal pH value tended to increase with *Spirulina* with an insignificant difference among treatments (P>0.05) being 6.70-6.92. Ruminal TVFA's concentration increased significantly (P<0.05), however, ruminal NH_3-N concentration decreased significantly (P<0.05) with the two levels of *Spirulina*. Group 3 showed significantly (P<0.05) the highest TVFA's concentration and the lowest NH_3-N concentration followed by G_2, while G1 had the opposite values. Similar results obtained by **Zhang (2011).**

Table 4. Rumen fermentation activity for the experimental dietary treatments.

Item	Experimental treatments			SEM
	G1	G2	G3	
pH value	6.70	6.83	6.92	0.05
TVFA's (meq/100 ml)	15.91^{c}	18.04^{b}	19.33^{a}	0.54
NH_3-N (mg/100 ml)	20.09^{a}	18.46^{b}	16.67^{c}	0.50

a, b, c: Values in the same row with different superscripts differ significantly (P<0.05).

Blood biochemical parameters

The effect of *Spirulina* additive on blood plasma biochemical parameters are presented in Table (5). Total protein concentration was significant higher with the high level of additive (G3) than that of the low level (G2) and control (G1), with non-significant difference between the two latter treatments. Respecting globulin concentration, the value of G3 was insignificant higher than G2 and significant higher than that of control group, with no significant difference between the two levels of supplement. Also, AST enzyme activity was decreased insignificantly with the low level of *Spirulina*-ration and significantly with the high level, compared with control (G1). Otherwise, each of albumin concentration and ALT activity did not affected by dietary treatments. These results agreed with those obtained by Malau-Aduli and Holman (2015) who found that AST concentrations were the lowest with high *Spirulina* supplementation levels. In addition, other researchers proposed that *Spirulina platensis* may have an effect on the increments in plasma total protein, albumin and globulin values (Mariey *et al*., 2014).

Table 5. Some blood plasma biochemical parameters for the experimental dietary treatments.

Item	Experimental treatments			SEM
	G1	G2	G3	
Total protein (g/dl)	7.53^{b}	7.77^{b}	8.30^{a}	0.22
Albumin (g/dl)	3.87	3.70	3.87	0.07
Globulin (g/dl)	3.67^{b}	4.07^{ab}	4.43^{a}	0.24
AST (U/L)	61.33^{a}	54.00^{ab}	41.00^{b}	4.06
ALT (U/L)	22.67	22.00	18.67	0.89

a, b: Values in the same row with different superscripts differ significantly (P<0.05).

Table 6. Blood haematological parameters for different experimental dietary treatments.

Item	Experimental groups			SEM
	G1	G2	G3	
WBC's (x10^{3}/µl)	9.07^{b}	10.63^{ab}	11.30^{a}	0.41
LY (x10^{3}/µl)	5.26^{b}	6.18^{ab}	7.15^{a}	0.33
MO (x10^{3}/µl)	1.22^{c}	1.63^{a}	1.46^{b}	0.08
GR (x10^{3}/µl)	2.59^{b}	2.83^{a}	2.69^{ab}	0.09
RBC's (x10^{6}/µl)	5.13^{b}	5.32^{ab}	5.58^{a}	0.08
HGB (g/dl)	8.73^{b}	9.13^{a}	9.20^{a}	0.18
HCT %	26.80^{b}	29.15^{a}	29.27^{a}	0.27
MCV (fl)	51.93^{b}	55.50^{a}	56.93^{a}	0.81
MCH (pg)	16.17^{b}	17.87^{a}	17.17^{ab}	0.36
MCHC (g/dl)	28.97^{b}	31.07^{a}	31.17^{a}	0.22
RDW %	13.57^{b}	14.60^{ab}	15.00^{a}	0.12
PLT (x10^{3}/µl)	246.67^{b}	268.00^{a}	272.33^{a}	11.21
PCT (µg/l)	0.134^{b}	0.143^{b}	0.168^{a}	0.01
MPV (fl)	5.70^{b}	6.00^{ab}	6.60^{a}	0.22
PDW (fl)	6.17^{b}	7.07^{b}	9.13^{a}	0.52

a, b, c: Values in the same row with different superscripts differ significantly (P<0.05).

WBC's= white blood cell count, LY= lymphocytes, MO= monocytes, GR= granulocytes, RBC's= red blood cell count, HGB= haemoglobin concentration, RDW= red blood cell distribution width, HCT= haematocrit, MCV= main corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration. PLT= platelet count, MPV= mean platelet volume, PDW= platelet distribution width and PCT= procalcitonin.

Blood haematological parameters

The present investigation showed that *Spirulina* algae that used as feed additive for cows caused a significant increase (P<0.05) in haematological parameters (Table 6). Among the different experimental treatments, G3 recorded the higher (P<0.05) haematological values in respect of (WBC, LY, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, PCT, MPV and PDW), while G2 had the best values (P<0.05) respecting MO and GR, compared to control one. Haematological studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. Haematological parameters are closely related to the response of animal to the environment, an indication that the environment where fishes live could exert some influence on the haematological characteristics (Gabriel *et al*., 2004). The increased WBC production may be due to the presence of phycocyanin and polysaccharides components in *Spirulina.* The WBC counts and Hb concentration were increased with supplementation of the polysaccharide of *Spirulina* in mice (at a dose of 30-60 mg/kg) and dogs (at a dose of 12 mg/kg) (Zhang *et al*., 2011).

Live body weight changes and body condition score

Live body weight and body condition score during the first 120 days of lactation season are shown in Figs. (1&2). *Spirulina* improved significantly (P<0.05) live body weight and body condition score, which decreased gradually until reach 90, 75 and 60 days and increased thereafter for G1, G_2 and G3, respectively. Live body weight changes were -53, -44 and -37 kg and body condition score change were -0.98, -0.88 and -0.70 for G1, G_2 and G_3, respectively. *Spirulina* increased live body weight by 3.82 and 6.58% for G2 and G3 compared to G1, respectively. The corresponding values for the body condition score were 7.19 and 16.01%, respectively. These results may be attributed to that *Spirulina* increased feed intake and improved energy balance during the transition period (Table 3). Results here are in agreement with those obtained by Kulpys *et al*. (2009) who found that dairy cows receiving *Spirulina* have been found to have improved body condition (8.5-11%) when compared to others receiving no *Spirulina.* It has been established that the stage of body condition at calving period is a crucial index as predicting milk productivity during the next lactation. Cows scoring less than 3.0 at calving produce less milk (Wallace, 2000). To achieve higher milk production, changes in body condition should be controlled. If the body condition at calving matches the standards, and in the first weeks after calving the rations are properly balanced, it would be possible to avoid decrease of milk production (Dechow *et al*., 2002).

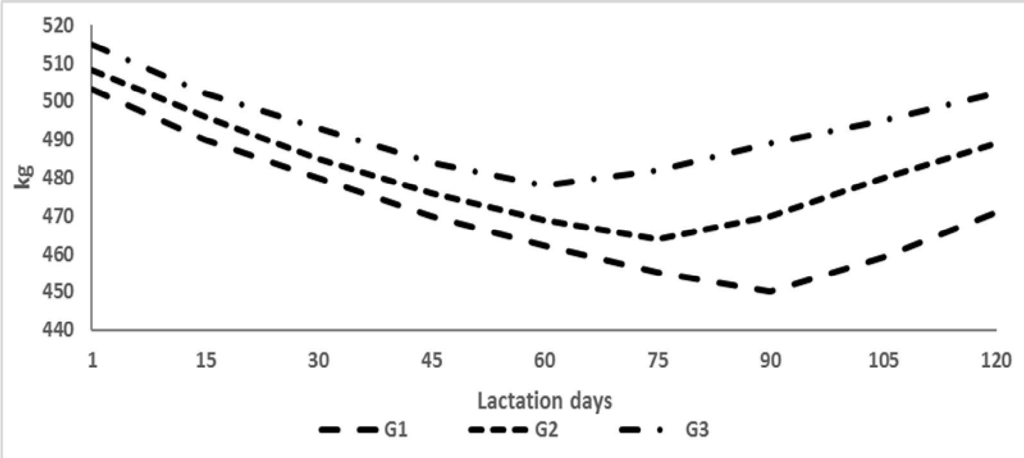

Fig. 1. Live body weight of cows in different groups during the lactation period.

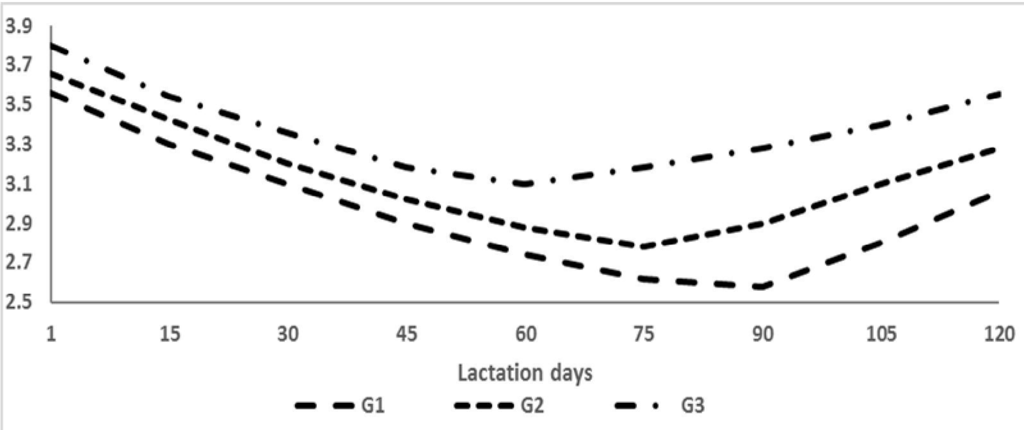

Fig. 2. Body condition score of cows in different groups during the lactation period.

Milk yield

Results of milk yield are shown in Table (7) and Fig. (3). Daily yield of actual milk and 4% FCM were increased significantly (P<0.05) with *Spirulina* addition, where G3 (high *Spirulina* level) recorded significantly (P<0.05) the highest daily actual milk and 4% FCM yield followed by G2 (low *Spirulina* level), while G1 (without additive) had the lowest yield. Actual milk yield of G2 and G3 increased by 8.01 and 14.73% compared to G_1, respectively. The corresponding values for 4% FCM were 14.12 and 26.57%, respectively. *Spirulina* addition improved milk persistency in which the peak period extended from day 45 until day 75 of lactation for cows in G1 and G2, while in G3 extended from day 45 until day 90 of lactation. The percentages of decreasing milk yield were 22.48, 17.14 and 12.33% of peak yield for G1, G2 and G3, respectively. These results agreed with those obtained by Kulpys *et al.* (2009) who found that cows receiving dietary *Spirulina* had a 21% increase in their milk production. The positive influence on the productivity of the tested groups could be due to the chemical composition of *Spirulina platensis* and its contents of biological micro nutrients, that potentially have vital role in most metabolic process in the cow's body and effectively increasing the milk production. According to literature (Simkus *et al*., 2008), *Spirulina platensis* consists of alkaline elements and other substances which can change the cattle fore-stomachs reaction to the alkaline side. That is highly significant because the activity of fore-stomachs micro-organisms and the fermentation process is the most effective when the fore-stomachs reaction to the inner environment is neutral. It is also crucial that enough volatile fatty acids are formed during the fermentation process because they are necessary for cow organism functions and milk synthesis.

Colostrum composition

Colostrum composition for different groups was presented in Table (8). *Spirulina* significantly (P<0.05) improved colostrum composition where G3 showed the highest percentages of fat, protein, lactose, SNF and TS followed by G2, while G1 had the lowest percentages. Moreover, the percentages of fat, protein, lactose, SNF and TS decreased from the first to the third day after calving. There is little evidence that nutrition within the range of diets typically fed to dairy cows, has much influence on either the volume or quality of colostrum. Black seed oil supplementation showed insignificant increase regarding total solid, fat, solid not fat, total protein, lactose and ash in buffalo colostrum during four days after parturition (Khattab *et al*., 2011).

Table 7. Average daily milk yield and composition for different experimental treatmens.

Item	Lactation period (month)				Mean
	1st	2nd	3rd	4th	
Actual milk yield (kg/day):					
G1	16.36c	18.07c	17.33c	16.11c	16.97c
G2	17.48b	19.39b	18.80b	17.65b	18.33b
G3	18.50a	20.61a	20.02a	18.80a	19.47a
SEM	0.27	0.31	0.30	0.32	0.18
Actual milk improvement %:					
G1	0.00c	0.00c	0.00c	0.00c	0.00c
G2	6.85b	7.30b	8.46b	9.55b	8.01b
G3	13.06a	14.06a	15.52a	16.70a	14.73a
SEM	1.54	1.70	1.62	1.57	1.00
4% FCM yield (kg/day):					
G1	14.88c	15.27c	14.96c	14.66c	14.94c
G2	16.62b	17.24b	17.11b	16.91b	17.05b
G3	18.24a	18.96a	18.95a	19.02a	18.91a
SEM	0.43	0.44	0.46	0.49	0.23
4% FCM improvement %:					
G1	0.00c	0.00c	0.00c	0.00c	0.00c
G2	11.69b	12.90b	14.37b	15.35b	14.12b
G3	22.58a	24.17a	26.67a	29.74a	26.57a
SEM	2.73	3.00	3.41	3.67	1.54

a, b, c: Values in the same column with different superscripts differ significantly (P<0.05).

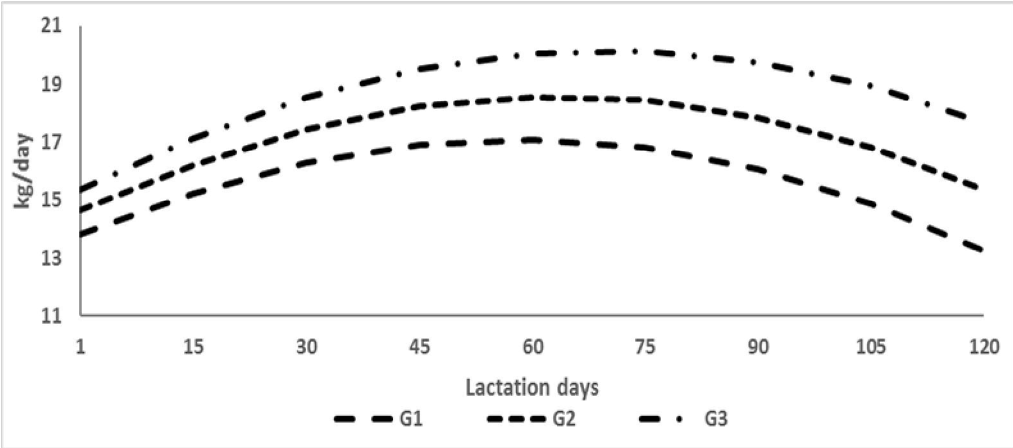

Fig. 3. Average daily milk yield for different groups during the lactation period.

Milk composition

Group 3 (2 ml *Spirulina/*kg LBW) showed significantly ($P<0.05$) the highest concentrations of fat, protein, lactose, solids not fat (SNF) and total solids (TS) followed by G2 (1 ml *Spirulina/*kg LBW), while G_1 had the lowest values (Table 8). The average increases in the contents of fat, protein, lactose, SNF and TS of G_2 and G3 were 10.28, 19.00; 8.47, 18.95; 8.73, 16.14; 7.90, 15.52 and 8.55, 16.50% compared to G_1, respectively. These results agreed with those obtained by Simkus *et al*. (2008) who showed an increase in milk fat (between 17.6% and 25.0%), milk protein (up by 9.7%) and lactose (up by 11.7%) in cows receiving *Spirulina* compared to those free from *Spirulina* supplement.

Electrical conductivity (EC) of milk

Electrical conductivity of milk showed opposite trend to milk yield, which *Spirulina* additive revealed significant ($P<0.05$) decrease in EC of cow's milk (Table 8). Group 1 (control) showed significantly ($P<0.05$) the highest EC value followed by G_2 (low *Spirulina* level), while G3 (high *Spirulina* level) had the lowest EC value. The EC values for G2 and G3 decreased by 4.19 and 7.38% compared to G1, respectively. The electrical conductivity of milk has been introduced as an indicator trait for mastitis over the last decade. The EC is determined by the concentration of anions and cations. If a cow suffers from mastitis, the concentration of Na^+ and Cl^- in the milk were increases, leading to increased electrical conductivity of milk from infected quarters. EC measurements in milk from healthy cows generally ranged from 5.5 to 6.5 mS (Norberg *et al*., 2004).

Table 8. Composition of colostrum and milk for different experimental treatments.

Item	Colostrum			Milk			
	1st day	2nd day	3rd day	1st month	2nd month	3rd month	4th month
Fat %:							
G1	8.01^{b}	5.79^{b}	3.59^{b}	3.40^{b}	2.96^{c}	3.09^{c}	3.40^{c}
G2	8.41^{b}	6.37^{b}	4.08^{b}	3.67^{ab}	3.26^{b}	3.52^{b}	3.72^{b}
G3	10.12^{a}	7.82^{a}	5.80^{a}	3.91^{a}	3.47^{a}	3.81^{a}	4.08^{a}
SEM	0.37	0.33	0.35	0.09	0.06	0.09	0.08
Protein %:							
G1	6.60^{b}	4.43^{c}	3.04^{b}	2.47^{b}	2.32^{c}	2.50^{b}	2.62^{c}
G2	7.43^{ab}	5.42^{b}	3.51^{b}	2.60^{b}	2.42^{b}	2.73^{a}	3.01^{b}
G3	8.26^{a}	6.46^{a}	4.48^{a}	2.98^{a}	2.60^{a}	2.88^{a}	3.35^{a}
SEM	0.30	0.31	0.24	0.08	0.04	0.05	0.08
Lactose %:							
G1	4.79^{b}	4.29^{b}	3.52^{b}	3.71^{c}	3.58^{c}	3.78^{b}	4.04^{c}
G2	5.83^{ab}	5.05^{ab}	4.32^{ab}	4.05^{b}	3.79^{b}	4.19^{a}	4.42^{b}
G3	6.60^{a}	5.32^{a}	4.61^{a}	4.30^{a}	4.10^{a}	4.37^{a}	4.78^{a}
SEM	0.31	0.21	0.20	0.08	0.06	0.08	0.10
SNF %:							
G1	12.09^{b}	9.45^{c}	7.29^{c}	6.88^{c}	6.59^{c}	7.01^{b}	7.37^{c}
G2	13.96^{a}	11.18^{b}	8.53^{b}	7.35^{b}	6.91^{b}	7.64^{a}	8.13^{b}
G3	15.56^{a}	12.49^{a}	9.80^{a}	7.99^{a}	7.40^{a}	7.96^{a}	8.83^{a}
SEM	0.57	0.48	0.40	0.14	0.10	0.12	0.17
TS %:							
G1	20.10^{c}	15.23^{c}	10.87^{c}	10.27^{c}	9.56^{c}	10.10^{b}	10.77^{c}
G2	22.37^{b}	17.54^{b}	12.60^{b}	11.01^{b}	10.17^{b}	11.16^{b}	11.85^{b}
G3	25.68^{a}	20.31^{a}	15.60^{a}	11.90^{a}	10.86^{a}	11.77^{a}	12.91^{a}
SEM	0.84	0.77	0.71	0.19	0.15	0.20	0.24
Ash %:							
G1	0.70	o.73	0.73	0.70	0.70	0.73	0.71
G2	0.70	0.70	0.70	0.70	0.70	0.72	0.70
G3	0.70	0.71	0.70	0.71	0.70	0.71	0.70
SEM	0.004	0.006	0.005	0.001	0.002	0.003	0.002
EC (mS/ml):							
G1				6.08^{a}	5.91^{a}	6.32^{a}	6.50^{a}
G2				5.95^{b}	5.77^{b}	6.18^{b}	6.35^{b}
G3				5.88^{b}	5.71^{b}	6.11^{b}	6.28^{b}
SEM				0.03	0.03	0.03	0.03

a, b, c: Values in the same column with different superscripts differ significantly ($P<0.05$).

Feed conversion ratio

Feed conversion ratio expressed as the amounts of DM, TDN, CP and DCP per 1 kg 4% FCM as affected by *Spirulina* additive are shown in Table (9). *Spirulina* led to a significant decrease (P<0.05) in the amounts of DM, TDN, CP and DCP per 1 kg 4% FCM, where G_3 recorded the lowest values followed by G2, while G_1 had the highest values. The improvements of feed conversion ratio with *Spirulina* may be attributed to the improvements in nutrients digestibility (Table 2), feed intake (Table 3), rumen fermentation activity (Table 4) and milk yield (Table 8). These results are in accordance with those obtained by El-Sabagh *et al.* (2014) who found that *Spirulina* supplementation in fattening lamb's diets improved feed conversion ratio, compared to the control group (P<0.05). Also, Mariey *et al*. (2014) reported that birds fed *Spirulina* diets achieved superior means of feed conversion ratio compared to those of the control group.

Table 9. Feed conversion ratio and economic efficiency for different experimental treatments.

Item	Experimental treatments			SEM
	G1	G2	G3	
Feed conversion ratio:				
DM kg/kg FCM	1.11^{a}	1.00^{b}	0.93^{b}	0.03
TDN kg/kg FCM	0.69^{a}	0.64^{ab}	0.61^{b}	0.01
CP g/kg FCM	134.54^{a}	121.57^{b}	112.49^{b}	3.10
DCP g/kg FCM	87.68^{a}	82.24^{ab}	78.42^{b}	1.67
Economic efficiency:				
Feed cost LE/day	33.78^{c}	35.39^{b}	37.00^{a}	0.38
Feed cost LE/kg FCM	2.34^{a}	2.18^{ab}	2.06^{b}	0.04
Total revenue LE/day	50.05^{c}	56.21^{b}	61.94^{a}	1.49
Net revenue LE/day	16.27^{b}	20.82^{a}	24.94^{a}	1.22
Net revenue improvement %	100.00^{c}	127.97^{b}	153.29^{a}	8.98

a, b, c: Values in the same row with different superscripts differ significantly (P<0.05).

The prices in Egyptian pound (LE) per ton were 2750 for concentrate feed mixture, 320 for fresh berseem, 350 for corn silage, 250 for rice straw, 2 LE/ liter fresh *Spirulina* with their media and 3.35 LE/ kg 4% FCM produced during year 2015-2016.

Economic efficiency

Data of economic efficiency presented in Table (9) showed that *Spirulina* additive resulted in significant (P<0.05) improvements in economic efficiency. Daily feed cost, total revenue of milk yield, daily net revenue and net revenue improvement increased significantly (P<0.05), but, feed cost per 1 kg 4% FCM was decreased significantly (P<0.05) with *Spirulina* compared to those of control. Group 3 recorded significantly (P<0.05) the highest average daily feed cost, total revenue of milk yield, net revenue and net revenue improvement, while G1 (control) had the opposite trend with all mentioned items. Net revenue of G2 and G3 increased by 27.97 and 53.29% compared to G1, respectively. Each 1 LE cost of *Spirulina* additive increased the income of milk yield by 6.02 LE. These results agreed with those obtained by Kulpys *et al*. (2009) who found that throughout the 90-day experiment, the average income from the milk of one cow from the experimental group was 378 LE or 21% more than that of controlled group. The use of cyanobacteria additives was economically effective because 1 LE costs for *Spirulina platensis* increased income from milk by 8.4 LE.

Reproductive performance

Postpartum reproductive parameters of Friesian cows as affected by *Spirulina* additive are shown in Table (10). *Spirulina* improved significantly (P<0.05) all the postpartum reproductive parameters. Cows in G_3 recorded significantly (P<0.05) the short periods from parturition until the first estrus and insemination, as well as days open followed by G2, while those in G_1 had the longer periods. Moreover, cows in G_3 showed significantly (P<0.05) the highest conception rate and the lowest number of service per conception followed by G_2, while those in G_1 revealed the opposite trend. These results agreed with those obtained by Mariey *et al*. (2014) who found that fertility percentage of eggs produced by two local strains of laying hens fed the *Spirulina*-containing diets were significantly superior compared to those of the control group. The investigations with different varieties of *Spirulina* have shown the positive influence on the female reproduction.

Table 10. Reproductive performance for different experimental treatments.

Item	Experimental treatments						SEM
	N	G1	N	G2	N	G3	
First estrus (day)	5	51.60^a	5	40.20^b	5	32.00^c	2.37
First insemination (day)	5	87.80^a	5	67.50^b	5	53.60^c	4.09
Days open (day)	3	129.60^a	4	92.70^b	5	73.60^c	6.43
No. service/conception	3	2.20^a	4	1.60^b	5	1.20^c	0.16
Conception rate %	3	60.00^b	4	80.00^{ab}	5	100.00^a	10.69

a, b, c: Values in the same row with different superscripts differ significantly (P<0.05).

CONCLUSION

Friesian cows with *Spirulina* and their media at the level of 2 ml / kg LBW in drinking water gave the best results concerning nutrients digestibility, feed intake, rumen fermentation activity, blood biochemical and haematological parameters, body condition score, milk yield, composition of colostrum and milk, feed conversion ratio and economic efficiency as well as post-partum reproductive traits.

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تأثير الاسبيرولينا على الأداء الانتاجى والتناسلى للأبقار الفريزيان

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استخدم فى هذه الدراسة عدد ١٥ بقرة فريزيان متعددة الولادات يتراوح وزنها بين ٥١٠– ٥٣٢ كجم فى مواسم الحليب من الثانى الى الرابع عند ٤٥ يوم قبل الولادة واستمرت لمدة ١٢٠ يوم بعد الولادة وقسمت الى ثلاثة مجموعات متماثلة بكل منها ٥ حيوانات. كانت المجموعة الأولى بدون اضافة (مجموعة المقارنة)، بينما أضيف طحلب الاسبيرولينا بالبيئة النامية عليها فى ماء الشرب الى المعاملات التجريبية المجموعتين الثانية والثالثة بمعدل ١ و ٢ مل لكل كجم من وزن الحيوان الحى. تم تنمية طحالب الاسبيرولينا على بيئة Zarrouk's المعدلة لمدة ٢١ يوم.

أظهرت النتائج ارتفاع معنوى فى هضم جميع العناصر الغذائية والقيم الغذائية فى المجموعة الثالثة عنها فى المعاملتين الأولى والثالثة وكانت كل القيم مرتفعة معنويا عنها فى المجموعة الأولى. زيادة المادة الجافة المأكولة غير معنوية فى المجموعة الثانية وزيادة معنوية فى المجموعة الثالثة بالمقارنة بالمجموعة الأولى. لم تتأثر قيمة درجة حموضة الكرش معنويا بالمعاملات، بينما زاد تركيز الأحماض الدهنية الطيارة الكلية معنويا مع زيادة اضافة الاسبيرولينا وأظهرت قيم تركيز نيتروجين الأمونيا اتجاه مضاد بين المعاملات. أظهرت معظم القياسات الكيميائية والهيماتولوجية زيادة ملحوظة مع زيادة مستوى الطحالب فى ماء الشرب للأبقار.

انخفاض وزن ودرجة حالة جسم الأبقار بعد الولادة حيث تقل تدريجيا حتى ٩٠، ٧٥، ٦٠ يوم ثم تزداد بعد ذلك فى المعاملات الأولى، الثانية، الثالثة على التوالى. سجلت المجموعة الثالثة معنويا عند مستوى ٠,٠٥ أعلى متوسط يومى لانتاج اللبن الفعلى واللبن المعدل ٤% دهن، تركيب السرسوب واللبن، معدل التحويل الغذائى والكفاءة الاقتصادية. فيما يخص الصفات التناسلية، سجلت المجموعة الثالثة معنويا عند مستوى ٠,٠٥ أقصرفترة من الولادة وحتى أول شياع، أول تلقيح، التلقيح المخصب، أعلى معدل خصوبة وأقل عدد من التلقيحات اللازمة للاخصاب.

نستخلص من هذه الدراسة أن اضافة طحلب الاسبيرولينا والبيئة النامية عليها بمعدل ٢ مل/ كجم وزن حى فى ماء الشرب للأبقار الفريزيان حققت أفضل النتائج فيما يتعلق بمعاملات الهضم، الغذاء المأكول، نشاط تخمرات الكرش، بعض صفات كيمياء وهيماتولوجى الدم، درجة حالة الجسم، انتاج اللبن، تركيب السرسوب واللبن، معدل التحويل الغذائى، الكفاءة الاقتصادية وكذلك بعض الصفات التناسلية بعد الولادة.